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Whitlam, Connor and Cameron

AUSTRALIAN scientists have been through a deeply traumatic period, as Peter Pockley reports (p. 448), and it is by no means clear that the somewhat ambiguous statement of Mr Whitlam on July 1 will be the end of the matter. The issues at stake deserve to be understood internationally, if only because they exemplify the sort of problems that are bound, sooner or later, to beset a country whose policy for the support of science has placed emphasis on unity by means of the large, some say monolithic, CSIRO (Commonwealth Scientific Industrial Research Organisation) with 6,600 staff spending £50 million annually, and a ministry trying to coordinate science.

CSIRO has traditionally enjoyed a high degree of autonomy in the running of its affairs. It reports direct to the Minister for Science, not through the ministry's secretariat, and thinks of itself as 'scientists run by scientists'. Promotion tends to be more by merit than by seniority. All of this has made CSIRO scientists wary of their status; to be moved into the public service sector and to report into the bureaucracy of a sectoral ministry would, they believe, seriously alter their style of doing things. It would also choke the interdisciplinary links which have been carefully nurtured in the last few years.

By themselves such arguments might simply be seen as defences of a cosy *status quo* and if used in normal times would have to be offset against other considerations. But in this instance the removal of roughly 500 staff from the jurisdiction of the Minister for Science (Mr Cameron) to that of the Minister for Minerals and Energy (Mr Connor) has some more disturbing aspects.

First, the tasteless manner in which it was done gives no confidence that any type of consensus politics was being practised. To act unilaterally and hastily is, of course, Mr Whitlam's privilege as Prime Minister, but it is one which inevitably leaves a feeling that the multi-lateral discussion has had to be short-circuited because the logic doesn't stand up too well to examination.

Second, the ambitions of Mr Connor, although relatively little mentioned in the furore that followed the decision, are not those that endear him to many scientists. His low profile throughout the affair, his failure to speak out on his own reasons for wanting to annexe portions of CSIRO and his undoubted aspirations to run a very large and very Australian resources

empire have left him with few supporters in the scientific community, even though his fascination with technology is unquestioned.

Third, and undoubtedly most important, there is much despair that the careful exercises during the past two years of Mr Bill Morrison (former Minister for Science) in attempting to enunciate a science policy for Australia could be so easily ignored. Only in January 1975, Mr Whitlam, on introducing the White Paper establishing the Australian Science and Technology Council (ASTEC), harangued scientists on the need to take science policy more seriously. And yet this decision was made without reference to ASTEC, in the absence of any indication in last year's OECD report on Australian science policy that CSIRO should be carved up, and without even the chairman of CSIRO being notified in advance. It will be difficult to get Australians to give up their time to thinking about the governing of science in future. And if the Department of Minerals and Energy is allowed to annexe what it wants, this could be a precedent for similar acquisitions by at least half a dozen other departments who have scientific needs close to CSIRO's interests.

In the midst of all this, Australia acquired a new Minister for Science and Consumer Affairs in Mr Clyde Cameron. There could only be sympathy for him at first at arriving to find a burglary had just taken place. But since then he has been travelling and his first major speech in the new job was delivered in New Delhi to the Indian National Science Academy. It gives grounds for concern. He declares "... science and technology is now responsible for activities that are polluting and poisoning the world's atmosphere, ionosphere and even stratosphere. ... At the rate we are now moving, it will soon be possible to fix a timetable for the ultimate end of human life itself. ... Australia wants its scientists to do two things: one to concentrate on those areas which will improve the quality of human life, not just in Australia, but everywhere. And secondly, to [concentrate on] research that will enable Australia to help remedy the incalculable damage which the abuse and misuse of scientific knowledge has already caused to nearly every country in the world. ASTEC will direct its attention to these two objectives." ASTEC, CSIRO and the academic community are in for a difficult time ahead. □



international news

THE Australian Government and public, and not least the scientists themselves, have been amazed by the intensity and unanimity of the fight by CSIRO staff to retain the integrity of their organisation following the raid by the Minerals and Energy Department to take over the CSIRO's Minerals Research Laboratories and Solar Energy Energy Studies Unit. At the time of writing, a compromise appears to have been reached, albeit one open to alternative interpretations, and CSIRO has at least held its ground.

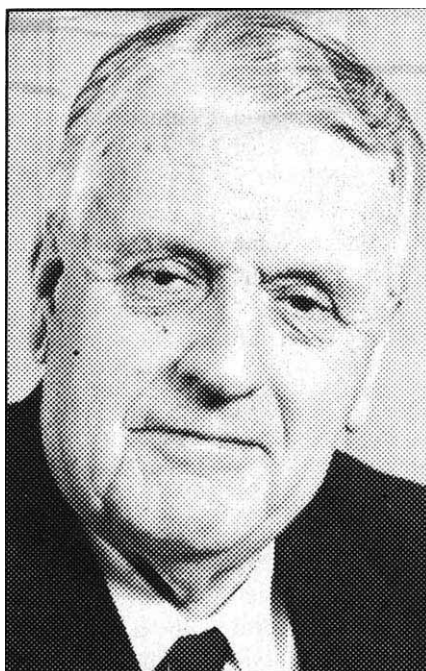
Among the many problems faced by the CSIRO Executive and supporters in the wider public in mounting their fight has been the simultaneous battle of Mr. Whitlam's government to survive in power. Politics in Australia has been turbulent in Labour's 2½ years in office, but the last few weeks have been remarkable for the severity of the battering taken by the government, mostly of its own making. No sooner had Mr Whitlam finalised his new ministry on June 6, by persuading Mr Clyde Cameron to accept the Science and Consumer Affairs portfolio (and, at the same time, announced amputation of CSIRO), than the byelection campaign began to go sour in the safe Labor seat of Bass in Tasmania vacated by Mr Lance Barnard whose resignation had precipitated the Cabinet reshuffle. Labor lost the seat in a landslide to the Liberals at a time when the CSIRO fightback was gathering great momentum.

The Prime Minister and his Minerals and Energy Minister, Mr Rex Connor, were hardly approachable on anything, let alone the CSIRO affair and the scientists' campaign was waged largely at arm's length against a silent adversary. Without a day's respite from their drubbing in Bass, the government was pushed into its greatest crisis when a long-simmering row about unorthodox ways of raising overseas loans of "petrodollars" (to the tune of \$4,000 million) came to a head. The bulky Mr Connor (who does not delight in his popular nickname of "The Strangler") and the nice but naive Deputy Prime Minister, Dr Jim Cairns (demoted from the Treasury to Environment), were the central targets of attack from the Liberal Opposition and the media.

Finally, Mr Whitlam sacked Dr Cairns, a drastic enough move in itself, but this was insufficient to keep the

CSIRO holds ground in fightback

from Peter Pockley, Sydney



Connor: hardly approachable.

wolves at bay, and Parliament was recalled for a special session. One of the dozens of members of Parliament and Ministers who came scurrying back from overseas to support their sides in the crisis debate on July 9, was Mr Cameron who had been visiting Delhi, Moscow and Paris, far from the maddening crowd of CSIRO staff. He had not been missed, though, for the Acting Minister for Science and Consumer Affairs, Dr Moss Cass, a quietly persuasive politician with a knowledgeable and sympathetic record on science policy matters, had taken up the scientists' case with drive and some effect.

Before Mr. Cameron arrived back in Australia, Mr Whitlam had issued a brief statement on July 1, which went some way to meeting the scientists' demands for autonomy in staffing conditions from the Australian Government Public Service. The Prime Minister said "that he wanted to make it clear that the fact that the Minister

for Minerals and Energy was responsible for those aspects of CSIRO's research work (minerals and solar energy) did not entail the transfer of the CSIRO's staff concerned to the Public Service. These staff would remain employed under the Science and Industry Research Act and it is not proposed that they should be transferred."

That this statement leaves much open to doubt is shown by the cautious statement issued the following day by the CSIRO Chairman, Dr Jerry Price, who has worked with great energy to defend his organisation. Dr Price said that CSIRO welcomed the announcement, and then went on to say: "The Executive of CSIRO is now examining the administrative machinery involved to put into practice the division of ministerial responsibility as outlined in the administrative arrangements order." For the first time CSIRO formally accepted that the administrative order "makes clear the movement of ministerial responsibility for matters relating to minerals and solar energy research from the Minister for Science and Consumer Affairs, Mr Clyde Cameron, to the Minister for Minerals and Energy, Mr R. F. X. Connor".

But, the puzzlement over how CSIRO can work to two Ministers remains. Mr Whitlam's announcement could be consistent either with CSIRO remaining intact as an organisation, or with a separate CSIRO-like organisation being formed, under the Minister for Minerals and Energy, from the transfer of the relevant CSIRO staff without change in their working conditions. The Minerals and Energy Department are understood to have worked on draft legislation to give effect to the latter alternative, but, to date, they have not showed their hand. Showing hands is not part of that Department's style, and in any case their energies have been consumed by the defence of their Minister and their equally redoubtable Departmental Head Sir Lenox Hewitt, who, apart from the tragic Dr Cairns, have been the central figures in the row over the petrodollars loans (which never were—such is the fate of unsuccessful borrowers).

Mr Connor could also obtain a stake in CSIRO's operations through nominating members of the Executive—the next full-time vacancy will arise in January 1976, but whether Mr

THIS account would be incomplete without a survey of the two years leading up to the raid on CSIRO. This story is only beginning to emerge as CSIRO collectively gains confidence to speak a little louder than before the crisis.

Mr Connor is one of the few Australian politicians to proclaim a personal interest in science. Modern technology is said to fascinate him deeply—his vision of a national pipeline grid, under public ownership, for the distribution of Australia's none-too-plentiful natural gas supplies has been one expression of his apparent belief that to apply technology on the grand scale is to control the development of society. Mr Connor and Sir Lenox Hewitt have shown single-minded dedication to their aim of coordinating, through government or legislative controls, all principal aspects of mining and energy policy, but their ways of achieving these ends have not been marked by their encouragement of public information and discussion. Perhaps they planned from the outset that gaining the control and exercising close direction of major research facilities in the minerals and energy fields were essential elements of their strategy—in many ways a sensible aim if considered in isolation—but they played their cards close to their chests.

In 1973 (the first year of Labor rule) mining appeared to be out of favour with the government. Overseas ownership and "rape of national resources" were the bogies. The CSIRO leadership, struggling hard to read the new government's mind and not finding itself closely in tune with the Science Minister Mr Morrison, decided to slow the rate of growth of their minerals research effort. This effort had flourished in the late 60s in the wake of Australia's mining boom; CSIRO had created new mining-oriented Divisions, such as Mineral Physics, and had grouped old and new Divisions together as the Minerals Research Laboratories under a Director, Mr Ivan Newnham.

Research into energy was partly located in these laboratories, for

example, investigations into coal, but such areas of work were largely dispersed throughout the CSIRO organisation. However, a separate Solar Energy Unit was formed, under Mr Roger Morse, early in Labor's first term of office, a move popular with Mr Morrison and the environmental lobby. This was a modest operation, and Mr Connor was able, unbeknownst probably to CSIRO, to start building a case for a single minerals and energy research organisation, a case which he may have thought politically unbeatable because of the brakes put by CSIRO on minerals research (demonstrable from budget figures) and the organisation's supposed neglect of energy research (demonstrable from the CSIRO establishment chart and some inside information).

From early 1973 CSIRO began to make a series of formal approaches to the Minerals and Energy people seeking joint meetings between senior researchers to define areas of cooperation and rationalisation for the achievement of government objectives. The reception was cool—in the case of the original letter no acknowledgement was received for six months, in marked contrast to the response from other new Departments established under Labor with whom scientific cooperation was rapidly and substantially put into effect, e.g. with Environment, and Urban and Regional Development.

Within a year, CSIRO convened its own in-house conference on solar energy with papers from researchers both at the centre and periphery of problems of energy conversion and storage, ranging from photosynthesis to wind machines and low grade heating devices. Minerals and Energy gained the list of delegates and their topics, and later (after the June 5 announcement) used it as a check list on CSIRO's willingness to relinquish their staff in these areas.

Meanwhile, the OECD examiners arrived to analyse Australia's science and technology policies in March and April 1974. They were given extensive cooperation by the Ministers and Departments of Science, Health and En-

vironment, but they let it be known that Minerals and Energy did not seem to welcome them, Mr Connor and Sir Lenox Hewitt are reported to have declined to see the OECD team who, in their August 1974 report, recorded that they were unable to identify any energy policy at the national level. This bald statement was well considered by the politically oriented OECD people, but it drew a vitriolic Parliamentary attack on their report from Mr Connor, which at least had the virtue of spelling out what the Minister believed to constitute a policy for energy.

By September, Mr Connor had begun to show his hand. He wrung a joint statement out of Science Minister Morrison to the effect that Minerals and Energy was "to become involved in the implementation of the results of solar energy research". While CSIRO would continue its research in low-grade heat conversion, Minerals and Energy "would take over the development phase of those CSIRO research results which were approaching practical realisation".

Around this time, the Minerals and Energy Department asked that CSIRO provide a list of staff working in the minerals and energy field (they already held what they believed was the solar energy part of this list). For this purpose, Sir Lenox Hewitt paid a visit to the Canberra headquarters of CSIRO; Dr Price, the Chairman, was absent and he was given no satisfaction by the two members of the Executive who received him.

By November 1974, some contact was restored but not at the level of the CSIRO Executive. Discussions, which the Executive later said they thought were of an informal, exploratory nature leading to possible liaison, were held between the Director of the Minerals Research Laboratories and Minerals and Energy. By this time, Professor Harry Messel, an Atomic Energy Commissioner, had become Mr Connor's adviser on nuclear and other scientific matters; he became involved in the build-up to the Prime Minister's announcement on June 5, which so stunned the CSIRO staff.

Whitlam's government can survive until then is now open to daily speculation. Even if the government survives, Mr Connor's power base may be eroded because of his part in the loans crisis.

When the next history of CSIRO is written, the researchers will have unaccustomed ease in obtaining documents for the 25 crucial days for CSIRO spanning the period June 5 to July 1, the dates of the Prime Minister's two statements. The official communication network and the un-

official grapevine within the organisation worked overtime to keep the scattered staff up-to-date with latest developments.

The press came to realise that this aspect of science policy at least was big news—probably the first time in Australian history that science policy has rated front-page and editorial treatment. Australian scientists, notable for their caution about dealing with the press (there are no full-time science correspondents on Australian papers), began to brief journalists like long-lost

allies. The crisis created a flood of general information about CSIRO which should never have been so cautiously damned up for so long. But, while the press were remarkably generous in their coverage of the CSIRO side of the story, it was notable that the reporters who covered it—necessarily only political and general journalists—were struggling to find in their memories or files any arguments about scientific achievements to support their reports.

In passing, it should be noted that

whatever the final outcome, CSIRO's approach to its publicity must now surely change to a more open, if not aggressive stance. Some (but by no means all) of CSIRO's present troubles with the government could have been averted by a more accurate reading of the political climate over the past two-and-a-half years. On the other side, some of the government's embarrassment from a public confrontation with an essentially non-political body like CSIRO could have been avoided by a better understanding of the nature of scientific endeavour and researchers themselves. Facts about Australia's achievements and potential in research are not common currency, even among local scientists, let alone politicians and bureaucrats. Each side of the confrontation has lacked information and comprehension of the other. Despite its debilitating effects on the short-term, then, it is possible that the open fight over 25 days did a power of good in bringing political realities sharply home to the scientific community.

Once placed in the fire, CSIRO staff learned fast the tough business of political pressure. For instance, once they noticed that the government-financed Australian Broadcasting Commission, despite a swag of science programmers on its staff, had given scant attention to the CSIRO story at a time when the press were headlining it daily, direct pressure was placed on the ABC hierarchy. A special radio programme followed, with unanimous statements of support for keeping CSIRO intact from some unlikely but influential sources. As implied already, CSIRO is not immune to criticism, but not a whiff of this emerged in the public debate.

Reportedly, Mr Connor, Sir Lenox Hewitt and their adviser Professor Harry Messel (of Sydney University) had been assured during the planning of their raid on CSIRO that the Minerals Research and Solar Energy staff subject to transfer to the Minerals and Energy Department would welcome the change. (It is difficult, at this stage, to be certain of the origin of this advice, but fingers have been pointed sharply in only one or two directions.) That such advice was catastrophically wrong, and that the CSIRO staff have a solid if quiet pride in their organisation, has been shown by the complete lack of statements of support for the Connor/Hewitt/Messel move from within or without CSIRO. Such was the anger generated among some CSIRO people that there were underground moves to turn the raid back on the raiders. It is remarkable that, if there were good arguments in favour of consolidating all minerals and energy research, none have emerged publicly, even from the Minister involved or,

indirectly, by the much-favoured technique of off-the-record leaks to the press.

From June 12 onwards, meetings in laboratories and groups of laboratories regularly drew hundreds of staff from across the board of CSIRO employment barriers, namely research scientists, technical officers and administrative support staff. The respective unions were at one on the issue. They decided to take strike action on June 18, but this was deferred at the last moment (as was a later stop-work meeting) by reports that negotiations were in hand to restore the *status quo*. There was, though, considerable uneasiness among the staff for they had already been advised from their Head Office that "discussions are proceeding to identify the officers, employees and physical facilities that will be so transferred" (to the Department of Minerals and Energy). In a meeting on June 10, Mr Connor had left Dr Price and Mr Cameron without any doubt that he expected the transfer to occur.

Dr Price went public the following day with a press conference, and by June 16 the CSIRO Executive had briefed the Acting Minister for Science, Dr Cass, that the transfer of staff and facilities from CSIRO was "wholly unacceptable" to CSIRO. Dr Price does not find public debate easy; he is essentially a quiet person, but he showed that if scientists are slow to ignite, they can be devilishly long in burning.

Attempts by Dr Price and the CSIRO Advisory Council to see the Prime Minister failed, but Dr Cass had some effect with a well-publicised telegram to Mr Whitlam. The CSIRO Officers Association took out a writ in the High Court seeking to block the transfer on grounds of illegality related to their much prized working conditions which are independent of strict public service rules. On June 18, the 40-odd Chiefs of CSIRO Divisions had met in emergency session—always some achievement in Australia due to the distance and cost of travel to Canberra. The Chiefs unanimously (including the various Minerals Research Chiefs) telegraphed a stinging protest to Mr Whitlam. On June 20, a massive petition gathered hurriedly from 4,000 CSIRO staff, from technicians through to Chiefs and Executive, was presented to the Prime Minister's office. Six days later, the staff took out a large advertisement in the national press, timed to precede by two days the Bass byelection.

The points common to all protests included the arbitrary manner of handing down the decision without previous consultation, the value of CSIRO's unique model of organisation, its formula of freedom from political interference, and concern for morale.

Appeals to the 1974 OECD report on Australian science which recommended that CSIRO be kept intact were added to requests that the question be referred to the new Australian Science and Technology Council.

Genetic manipulation and the WHO

THE involvement of the World Health Organisation in the controversy that at present surrounds genetic manipulation experiments is welcome for a number of reasons, not all of them immediately obvious. It is true that Dr Halldan Mahler, the WHO's Director General, has not himself made a direct statement of the position he is taking on behalf of the Organisation. But the publication of the recommendations made to him by his Advisory Committee on Medical Research (ACMR) is sufficient indication of the firm intention to become involved, the more so since one of these recommendations suggests that "the Director General pursue appropriate means" for publicising the committee's conclusions on this subject.

The hazards involved in genetic manipulation, the need to ensure that every possible precaution is taken in controlling research, and the importance of keeping the public aware and as well informed as possible, have already aroused discussion and violent debate worldwide, and such matters were already high on the agenda of this year's meeting of the ACMR. It is to that committee that the Director General would naturally turn for advice, in particular in the form of a lengthy and comprehensive statement from Dr Joshua Lederberg of Stanford University School of Medicine.

Extreme opponents of the work in this field will certainly be concerned at the very positive nature of the committee's first recommendation, which is to the effect that "The continuation (under appropriate safeguards) of microbiological research, including genetic manipulation and cell fusion studies, is of the utmost importance for progress in medicine and public health".

Other recommendations make it quite clear that the committee sees the WHO as playing a leading part at the international level, in keeping all concerned—in the scientific community as well as governmentally—continually aware of developments in this field and of the implicit dangers. On a more positive note, priority should be given to research aimed at the development of the safest possible biomaterials for such research, and to the evolution of techniques that reduce the hazards of working with them. Internally, the

WHO itself is recommended to co-ordinate all activities (at present scattered among a number of different departments) dealing with subjects in any way connected with this field. The organisation, too, will need to establish itself as "the international focus for information and its dissemination at the national level; "collaborating centres" for work in this field should be designated; and the WHO will need to play a leading part in the training and inter-country liaison that must be part of the essentially global approach to research and development of this kind. □

UNESCO science in S. E. Asia

from Yoshinobu Kakiuchi, Tokyo

AT its 17th General Conference UNESCO adopted a resolution aimed at the promotion of research and advanced training on the basic sciences with special emphasis on the needs of developing countries, and to this end UNESCO headquarters in Paris and the Field Science Office for South-East Asia in Jakarta have been working with the Japanese National Commission for UNESCO.

The meeting on regional cooperation in basic science in South-East Asia was held in Tokyo last year to consider approaches, and scientists from nine countries from South-East Asia (in-

cluding Korea and Japan), and observers from Australia and New Zealand and representatives of international organisations joined the meeting. Taking the survey report prepared by two UNESCO consultants into consideration, the meeting agreed to select the field of the chemistry of natural products including microbiology as the first approach to the project.

The second meeting on regional co-operation was held in March this year in Tokyo and Osaka, and the meeting confirmed the establishment of the "Regional Network for Microbiology of Natural Products in South-East Asia" and the name of the interim points of contact of each participating country (a university involved in studies in the field) was suggested by the participant representing the country. The meeting also scheduled a meeting some time in late 1975 to discuss a more detailed picture of the similar network in chemistry, the establishment of which has already been agreed upon in the first meeting.

UNESCO is trying to mobilise all possible means available within the existing framework of its regular budget, but there is a need for more resources for this purpose. Japan has agreed to contribute cash in the form of a fund-in trust designed for use for specified objectives. There has been a fairly long experience in Japan of running UNESCO international graduate courses, one for chemistry and the

other for microbiology (this is actually a one year course for foreign students, not necessarily those from Asian countries) and Osaka University is operating the course for microbiology, with the full consultation and cooperation of microbiologists from universities and research institutions all over Japan. Japanese scientists recommended Osaka University to serve as the Japanese point of contact, after consultation with Professor Kei Arima of University of Tokyo, who is at present a member of the International Cell Research Organisation (ICRO).

For the fiscal year 1975, Japan contributes \$50,000 in the form of fund-in-trust, and also donates approximately \$18,000 equivalent of research equipment. Similar contributions will be expected in 1976. Funds and equipment are to be used exclusively for the academic activities of the network both in microbiology and chemistry, but the amount is by no means sufficient.

At the last Tokyo-Osaka meeting, Mahidol University in Bangkok, Thailand, agreed to serve as the regional centre for microbiology. It also functions as the national point of contact of Thailand, and Professor Pornchai Matangkasombut, who is in charge of research in microbiology in Mahidol University, is at present heavily involved in detailed planning of the structure and activities of the regional network. □

"It is undoubtedly a misnomer to speak of a British technological strategy". That's the harsh verdict of Dr Robert Gilpin, Professor of Public and International Affairs at Princeton, delivered in a mammoth report to a Congressional committee last month. Gilpin suggested that for various reasons, the British "on the whole have made very poor use of their rich scientific and technological resources", and he warned that the United States may make the same mistakes.

Gilpin's observations on Britain's technology policy were contained in a special report to the Joint Economic Committee on the hoary problem of how to integrate science policy and economic policy. Noting that "underlying the British malaise has been the problem of making the adjustment from the status of a global imperial power to that of a middle-sized European state", Gilpin suggested that the relative industrial decline of the United States poses similar problems to that country. The US, he suggested, should learn from the mistakes of the British.

"In the first place", Gilpin suggests, "British Government expenditures, like

those of the US have been overly concentrated in a relatively few areas such as defense, space and atomic energy. The government has taken upon itself the role of entrepreneurship and has concentrated upon commercial development instead of on research, exploratory development and related activities. In substituting its judgment for that of private entrepreneurs with respect to the commercial 'ripeness' of particular high technology projects, the government has assumed a responsibility and tasks which governments do not do well. As a consequence of this neglect of the market very few of these costly projects have had commercial success".

Gilpin goes on to suggest that "the British government in a number of significant cases has made commitments to full-scale commercial development of particular technologies too early and on too big a scale" and notes that "there has been a neglect of more traditional sectors of the economy which for historical and institutional reasons tend to under-invest in R & D."

Finally, he suggests that "the British have failed to integrate sufficiently the

three estates of science and technology; universities, government and industry. They have failed to create the necessary mechanisms to bring together the sources of new scientific and technical knowledge and the industrial users of knowledge. A disproportionately high fraction of British research and development has been conducted in government laboratories or in industry-wide cooperative laboratories catering to specific industrial sectors . . . While this latter set-up has served to improve the state of the several technical arts . . . the spill-over of government-supported military, and related research into the private industrial sector has been minimised".

The underlying failure of British science and technology policy, Gilpin asserts, is that "the government has tried to supplement rather than complement the private market . . . As a consequence, although the British are among the most technologically rich and resourceful people in the world, they have been unable to harness these resources to generate a sufficiently high rate of economic growth and competitive imports".

Canadian science: the golden era

from David Spurgeon, Ottawa

In his final annual report as chairman of the Science Council of Canada, Dr Roger Gaudry looks back to a "golden era" of science policy in 1966, when the council was created, and forward another nine years to the problems of 1984. And in passing he comments on the crises of today, in which "the comfortable academic debate over science policy has largely become a thing of the past for most of us."

The former rector of the University of Montreal, who recently took over as first president of council of the new United Nations University, was the Science Council of Canada's second chairman, after Dr Omond Solandt, the founding chairman. His terms expired this summer.

The "science policy era" had barely begun when the Council was created in 1966, Dr Gaudry recalls. The Glassco Royal Commission Report on Government Organisation was barely three years old, the Senate special committee on science policy had not yet been established, and Canada had not yet been investigated in the OECD series of national science policy studies.

Before 1966, the country's science policy was, in the terminology of the Senate committee report, a "hidden" one. This, said Dr Gaudry, was because the greatest portion of policy work was not close to the point of application and thus was of interest only to the scientific community; most federally-supported science was housed in government laboratories and the universities and was largely self-determined; and because federal funds were largely given without strings, their allocations were not politically important. "Rarely did science or science-related items become public issues," says Gaudry.

By 1966, however, three sets of issues related to science and technology surfaced in public discussions and the press: the controversy over four Big Science projects; arguments over the scale and distribution of growing national research and development expenditures; and discussions over mechanisms for policy formulation and implementation.

The four Big Science projects were: Atomic Energy of Canada Limited's plea for construction of an Intense Neutron Generator (ING), which was opposed by many in the academic engineering community; the withdrawal by the federal Department of Industry of support of McGill University's High Altitude Research Project (HARP); the federal Department of Energy, Mines and Resources' plan to

construct the Queen Elizabeth II Telescope in British Columbia, in the face of divided support from astronomers; and the proposal by three (later four) universities in western Canada to build a Tri-university Meson Facility (TRIUMF). The latter was the only one of the four projects destined to be funded to completion.

"Viewed in retrospect," says the Science Council chairman, "the mid-sixties were a golden era for science and technology, an era of enthusiasm, of rising budgets and of rising hopes. Canada's gross national expenditure on R & D was 15 per cent higher than in 1965, and that at a time when the annual rate of inflation was about 3.6 per cent."



Gaudry: looking back, looking forward

Science policy discussion of the era focused heavily on matters of research *per se*, as opposed to questions of the uses of science or the effects of technological programs, says Dr Gaudry. Even the Big Science projects had scientific, not technological goals. The participants were drawn almost exclusively from the scientific community itself.

By contrast, "the nature of today's discussion is both more technological and more political in character . . . many of the political issues of today revolve around what technology is, or is not, doing for us." The arguments now surround the James Bay Hydro Project, the Mackenzie Valley Pipeline, the Alberta Oil Sands Project, and the establishment of a second international airport near Toronto, at Pickering, Ont. And these arguments are over the validity of the economic objectives, the environmental impact and the costs, which are expressed in billions rather than millions of dollars.

"The adversaries in these discussions come from all segments of our society

rather than from the confines of the scientific community *per se*. What has changed materially, it seems to me, is the nature of the public's attitude to technological development. Our outlook has matured considerably, we realise that 'bigger' is not necessarily 'better', we have recognised the concept of external costs which can spring as side effects from major projects, and all in all we take a more circumspect view of potential technological changes. What remains to be seen is the extent to which scientific evidence will be given due weight in decision making."

Thus the perception of what science policy is all about has changed since 1966, as well as the substantive issues. Environmentalism, anti-economic growth thought, economic nationalism, and the emergence of the provinces as significant factors in technological areas, have all come to the fore.

In the background, there has been a small but articulate "anti-science" movement, and demands for technology assessment. Debate concerning industrial strategy has also arisen. And through it all runs the thread of social responsibility, "the growing recognition that our technologies can be made either to serve or to dominate our society."

Looking ahead, Dr Gaudry sees three international problems as important: population growth and inadequate food supplies; resource supply problems due both to depletion and the action of international cartels; the possibility of nuclear proliferation.

He suggests Canada should consider participating in international technological ventures, and says she should avoid being isolated in the face of increasingly organised trade blocs. Domestically, there will have to be some form of national long-range planning in order to solve some problems, such as that of energy supply.

Changes in the structure of the population will have to be coped with, as well as chemical assaults on the environment. Industrial strife may follow technological change if not handled properly, and more public attention may have to be paid to defence technology. And "we will be forced to pay more attention to the concepts of a 'conservative society' and will move away from the maxim that any 'demand' must be satisfied, irrespective of how the demand is generated."

On the technological front, Dr Gaudry sees growing concern over increasing dependence on systems so large that redundancy in them cannot be afforded. And on the basic research front, the moral and ethical dilemmas of progress in some areas of biology, particularly genetics, will influence policy. □

A PROTOTYPE British-built rice mill, costing £10,000, is on its way to Indonesia, where the government plans to tackle inefficiency in rice milling by introducing modern mills of a sufficiently small scale to suit the needs of the country's predominantly subsistence rural economy. At present Indonesia's crop is processed largely on a small scale, either by hand pounding or by milling in wasteful machines (the most widely used being a modification of the 19th century Engleberg coffee grinder, which only produces 500 kg of edible white rice—more than half of which is broken grain—from a tonne of paddy). However, official statistics rate milling efficiency at an inflated level of 60% probably because extrapolations from a few large, modern mills are applied to the total paddy crop, although, as a 1972 UNIDO survey pointed out, these mills only handle a small percentage of the Asian paddy harvest.

Rice constitutes the staple diet of more than a third of the world's population, in particular, of 90% of the low income families in the densely populated regions of tropical Asia. Discussions of rice production are sometimes obscured by the fact that—unlike the other staples, wheat and maize, with which rice is often bracketed—there is a substantial difference between the weight of the harvested, threshed and dried paddy, often called rice, and the final yield of edible milled rice. Avoidable mechanical damage during this milling process could be destroying 20–25% of the potential yield of edible rice from paddy, according to surveys carried out in Indonesia and the Philippines by British consulting engineers and agronomists, under the auspices of the British Agricultural Export

Council and the Department of Trade and Industry.

In the milling process, the inedible outer hull, which makes up about 20% by weight of the harvested dried paddy, is removed, and the final yield of edible white rice, separated from the bran—which makes up a further 10% of the paddy weight—is, ideally, 70%. Milling efficiency in the various countries of tropical Asia is said to vary between 60% and 70%, but some British experts believe, on the basis of observations in Indonesia and the

Grain gains

by Eleanor Lawrence

Philippines over the past few years, that the published figures for milling efficiency are over optimistic.

A large modern mill which deals with the paddy in several steps—hulling, separating the bran, and polishing the grain—can produce up to 700 kg of milled white rice per tonne but these large mills, capable of handling anything up to 100 tonnes of paddy an hour do not fit into the social pattern of a predominantly subsistence rural economy. The small farmer and his family, who in Indonesia, produce on average 1.5 tonnes of paddy from two crops on their half-hectare small-holding each year will eat the rice from about 1,200 kg of this, which will be milled locally or hand-pounded on the farm and will only send the surplus to the modern mills which are often far away and often inaccessible at certain times of the year.

The Indonesian government is now remedying this situation and has introduced a programme of small, more

efficient local mills which can deal with the paddy at all stages from the initial threshing of wet paddy from the straw. The mechanically-dried paddy can be stored in silos until needed. These mills will incorporate a more efficient huller, which is being developed in Britain at the moment, and would be able to recover about 65% of the paddy as edible milled white rice of good quality, as well as conserving the oil and protein-rich bran for use as an animal feed and producing clean hulls which will be used to power the mechanical driers (specially converted from oil-burners). With traditional milling, either in the Engleberg mill or by hand pounding, an unusable melange of hulls, bran and powdered rice (20%, 10% and 20% respectively of the original paddy weight) is left either to smoulder or to be dumped in nearby marshy land. Each mill would serve about four square miles bringing it within easy reach of all the farmers in the area.

For Indonesia, with an annual paddy production of upwards of 23 million tonnes, a real milling efficiency of 65% covering the total paddy crop would provide an extra 3 million tonnes of edible milled rice. A preliminary survey in the Philippines has also indicated that milling efficiency over the total paddy crop is much lower than officially assumed. If the situation in Indonesia and the Philippines is paralleled throughout tropical Asia, more than 16 million tonnes of edible milled rice are being lost each year in dust, several times the entire world exports of rice each year—a substantial contribution to the 'food crisis' endemic amongst the peoples of tropical Asia who need the rice only they can produce.

A little knowledge...

from Vera Rich, London

"EVERYONE has won", announced Mr Brezhnev at the end of the recent Helsinki talks on detente and arms limitation. According to the rules of Lewis Carroll's caucus race, everyone should therefore have prizes. But although the agenda of the conference included not only political and economic issues, but also human rights, the free flow of information between east and west, and the possibility for all citizens to travel freely, one group at least stands to gain no benefits from the Helsinki agreement—the ever-growing group of Soviet Jewish refuseniks. Hardly was the ink dry on the agreement, when the Moscow cyberneticist Aleksandr Lerner was informed "officially" that Helsinki will mean

"no change" in the rules governing the emigration of Soviet Jews.

The problems involved in visa applications are particularly acute for scientists, since it is easy for the authorities to claim that they have had access to classified information. With the new atmosphere of detente, this may lead to some curious anomalies. One such case is that of Aleksandr Druk, who was working on the electronics of the space programme before he applied for a visa and was subsequently dismissed some three years ago. Although he had never visited the Baikonur cosmodrome or even seen a spacecraft, he has been informed that the question of his emigration "will be decided only in 1980". Yet, as Druk himself has pointed out, the Apollo-Soyuz mission has led to the sharing of information of far more potential importance than any which

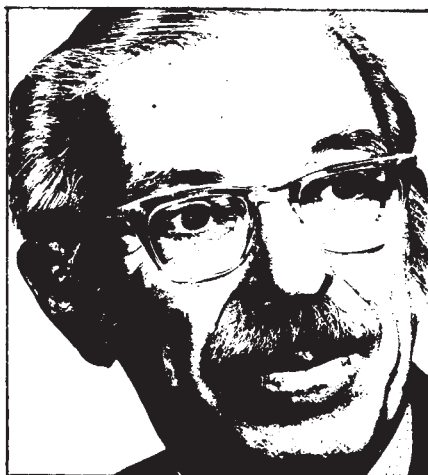
he could divulge to the West.

In other fields, too, the clamp down on Soviet Jewish scientists continues. A number of them are at present under considerable pressure because of their connection with the *samizdat* journal *Jews in the USSR*. This journal, founded by Professor Aleksandr Voronel (the founder of the "Sunday Seminars" for refuseniks), carried articles on historical, philosophical and religious topics, and was intended, according to Voronel, to explore the meaning of Jewish traditions and culture against the background of tacit discrimination in which Soviet Jews must live. A number of prominent scientists were connected with the journal; those under pressure at the moment include the physicists Aleksandr Lunts and Eitan Finkel'shtein and the cyberneticist Grigori Rosenshtein. □

It came to the table accompanied by the limp, succulent bacon and hard toast for which English breakfasts are famous, and it was so delicious that I bought a jar to take back to the States. The label said, "Contains Sugar, Oranges, No Preservatives, No Colouring". Now sugar is, of course, one of the most ancient and universal of food preservatives, and is certainly the one used in largest quantities. The colour of oranges makes them glow like lamps against the dark green leaves in the citrus groves of California. Colourless marmalade without sugar is difficult to imagine. The label was written, of course, to quiet the fears of those who are convinced that they are being harmed by food preservatives and food colouring; that they are hapless victims of malevolent concocters of artificial viands. These fears are even voiced by the Neuberger Report which says "Flavouring agents and other food additives may have harmful effects" (p. 6) . . . "Food additives may be converted into carcinogens" (p. 138). Presumably the authors were talking about nitrites, but generalised accusations are easy to make and difficult to pin down. Rainwater during a thunderstorm contains nitrites, too.

The skin of an orange is its protection against invaders. I have no idea whether orange oil would pass all of the elaborate safety tests that are used for new food additives but I think it is quite unlikely. It contains forty-two

Peel meal



THOMAS JUKES

chemical entities including 12 alcohols, 9 aldehydes, 2 esters, 14 hydrocarbons and 4 ketones. One of the entities is a phenolic compound called tangeretin. When tangeretin was injected subcutaneously into pregnant rats at a level of 10 mg per kilo of body weight, 83% of the offspring were born dead, or died within three days. Foods are tested for toxicity, of course, by feeding rather than by injection, but one can imagine what a hullabaloo would start if a "synthetic" food additive gave such a response. There are hundreds of "natural" components of foods that

have not been evaluated for toxicity in human subjects, and probably never will be. If we believe the assurances of analytical chemists that traces of a substance are present, we must also learn to accept the findings of toxicology that there are thresholds of toxicity that can be experimentally defined. But we tend to trust Mother Nature, and suspect the organic chemist, so that "synthetics" are examined much more stringently than natural compounds. Our lives are a curious mixture of rejection and acceptance of science, and this shows prominently in attitudes towards food.

Somehow there is a feeling that human beings have developed the ability to cope with injurious substances in common foods by an evolutionary process, but that a "new" chemical is quite likely to be unmanageable. This assumption is unwarranted as a generalisation. The process of alimentation consists of devouring plants and small animals that have always contained numerous "chemicals" that are more or less toxic. These are dealt with by excretion or metabolism unless the amounts ingested become too high for comfort. So, most literally, we may have a "gut feeling" that orange marmalade is a great food; and in this I would include even the less fancy brands to which extra amounts of citric acid and pectin, two normal ingredients of oranges, have been added. □

correspondence

The waiting game

SIR,—I wish to bring to the attention of all university departments the problems of one section of the scientific community; UK postdoctoral fellows currently engaged in research at overseas institutions, which I feel will add to the current discussion of the future structure of the UK university system. Other than the ridiculous practice of setting deadlines for the receipt of applications for university positions which expire before the particular advertisement reaches foreign shores, there is a much more serious problem involving the time frame.

In my own experience university lectureships tenable from October 1975 were advertised in your columns starting in May. Allowing three months for the receipt of applications and testimonials, the short-listing of candidates and finally the acceptance by one candidate, this means that perhaps several

hundred unsuccessful applicants are kept in limbo until August to hear their fate. Amongst these the applicants from overseas are often worse off because, due to visa problems they cannot retain their fellowships indefinitely. Often such candidates will also have applied to universities in other European countries where things are decided more rapidly; this can mean being faced with the decision to accept or reject a firm offer from, say a German laboratory, by the middle of July, or risk everything and wait upon the deliberation of the ponderous UK system.

It is apparent that to ensure that UK university appointments are made from the best pool of candidates available there must be radical changes in the hiring system. Since this would require advance information of budgets and staff needs, government and university cooperation is needed.

RICHARD JAMES
Princeton University, New Jersey

Close relatives

SIR,—Gold (*Nature*, July 10, 1975) has shown how two individuals (a mother and baby) may be differentially aged by a general relativistic effect. However, the method chosen involves the mother going out to collect masses from afar, since the act of 'going out' cannot be achieved without the mother experiencing an acceleration, then the mother like the baby will also age more slowly than an inertial observer situated at a large distance from the mass shell. Indeed, during her nightly travels it is possible for the mother to age more slowly than the baby.

I therefore question whether it is at all possible for a person to construct such a mass shell without experiencing acceleration.

Yours sincerely,

L. A. KING

24 Garston Crescent,
Calcot, Reading, UK

news and views

Mercury since Mariner 10

from a Correspondent

The First International Colloquium on Mercury was held at the California Institute of Technology on June 25–27, 1975. It was chaired by Bruce Murray of Caltech.

COMPARATIVE terrestrial planetology is an interdisciplinary field which has come of age as a result of satellite studies of the Moon, Mars and, most recently, Mercury. In the last decade, a number of complex, unmanned devices have investigated these objects and man himself has personally inspected one of them. The most remarkable result of these studies has been the identification in the photographs obtained of a universal process of cratering. The consensus is that most, if not all, craters are due to impact bombardment by planetesimals, some of which come from beyond the orbit of Uranus. Combined with the returned lunar samples, providing age dating, it now appears certain that early in the history of the formation of the Solar System, the terrestrial planets were subjected to an intense, cataclysmic bombardment process which has mainly shaped their surfaces unless they

possess substantial atmospheres. These matters and other new results concerning the innermost terrestrial planet, Mercury, were discussed and debated at the recent colloquium.

Understanding of Mercury is based primarily upon the USA Mariner 10 spacecraft results. They were obtained during an unprecedented celestial sequence of three successful fly-bys of the planet on 29 March 1974, 21 September 1974 and 16 March 1975. In view of current economic conditions, the space technology community can take great satisfaction in the multiple re-encounters achieved because they permitted a unique synergistic study of unexpected results obtained at first encounter. Any future return to Mercury seems unlikely for at least the next decade.

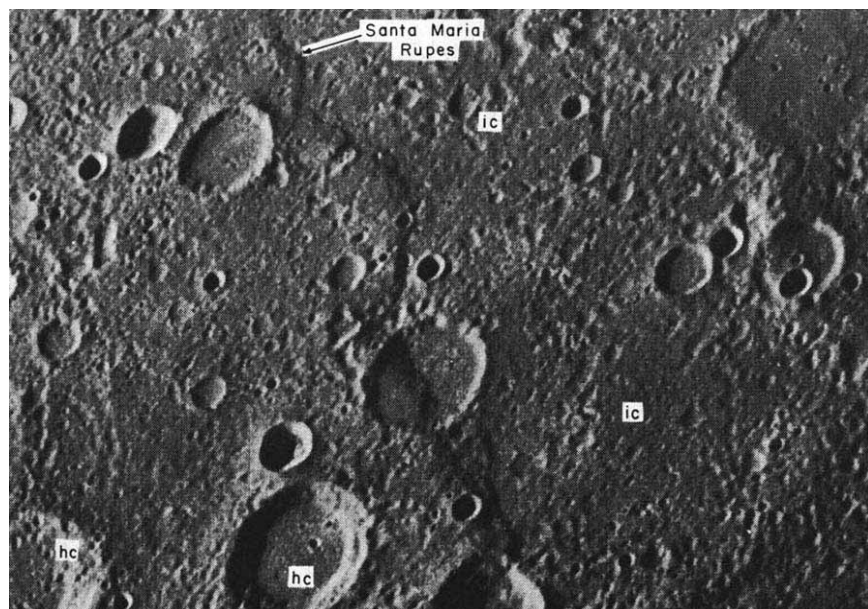
The most unexpected result of the first encounter was the identification of a strong solar wind interaction with the planet. This leads to the development of a detached bow shock wave as the solar plasma is deflected around the planet and the occurrence of intense bursts of energetic particles in the magnetosphere of the planet. The third fly-by unequivocally confirmed the

existence of the modest magnetic field (about 1% of Earth's intensity) and solar wind interaction. The consensus at the colloquium was that the field origin is most probably due to an active dynamo in the planetary interior. It was noted that from a magnetohydrodynamic fluid viewpoint, Mercury and its interior are rotating rapidly and thus could sustain such a dynamo.

In spite of its great success, it is doubtful if Mariner 10 will provide a precise measure of the moments of inertia and hence the distribution of mass within the planet. Nonetheless, the indirect evidence for a large, dense core seems compelling based upon theoretical models of the thermal history of the planet, global expressions of compressional features, and the existence of a modest magnetic field. The uncertainty in geochemical characteristics, especially of the radiogenic isotopes, leaves many degrees of freedom in predicting the present thermo-mechanical state of the interior. Some think that convection is inevitable in order to account for the unique synchronisation of the spin period and orbital periods of Mercury ($T_{\text{orb}} = 3/2 T_{\text{spin}}$). One specific issue unresolved is distribution of heat sources which would be responsible for forming a large core (70% of the surface radius) and maintaining it sufficiently fluid-like to permit convection up to the present time.

One of the significant results of the high fidelity photographic reconnaissance of Mercury was the identification of heavily cratered terrains and large basins readily evident in all photographs, the largest of these being Caloris with a diameter of 1,300 km! The development of a cratering chronology was one of the principal challenges to comparative planetologists studying impact bombardment of the terrestrial planets. The higher gravity on Mercury limits the radial extent of ejecta blankets and permits a more pristine view of the cratering sequence than on the Moon, with its lower gravity, or on Mars, where alteration by Aeolian weathering occurs extensively.

The extent of volcanic processes, in both space and time, was debated as offering the most logical explanation



Intercrater plains (ic) and heavily cratered terrain (hc) typical of much of Mercury outside the area affected by the formation of the Caloris basin. Abundant shallow elongate craters and crater chains are present on the intercrater plains. Prominent scarp Santa Maria Rupes cuts both intercrater plains and old craters. Scene is 200 km across.

of the smooth plains, wrinkle ridges and lobate scarps observed. Unquestionably to some, the plains areas appear filled as a result of internal activity. But yet how is it possible for younger craters to be floored with plains-like looking material which the cratering process should have obviously disturbed? The view was also expressed that the intercrater plains represent a process contemporary with the cratering and occur as a result of impact melting. The debate is reminiscent of the lunar questions in the mid-1960s following the early lunar orbiter photographs but prior to the Apollo landings.

It is readily admitted that it is not yet possible to consider development of a single model of evolution of this planet. In addition to its anomalously high average density, we now know its surface to be very Moon-like and yet with its modest magnetic field its interior must be more Earth-like. Where Mercury fits in the grand scheme of the origin of the terrestrial planets is not certain. It can reasonably be expected that future studies of these results and comparisons with the Moon and Mars (with the USA's Viking 76 landers) will shed light on this fundamental question.

Human cytogenetic registries

from a Correspondent

THE astonishing success of human cytogeneticists in communicating their discoveries in a standardised and comprehensible way is due to their early recognition of the need for a standard system of nomenclature. To this end, participants at four international meetings have made recommendations and devised systems of nomenclature, each of which has extended and modified the previous one and thus successfully kept pace with rapid technical advances. The meetings were arranged on an *ad hoc* basis and had no authority save their broadly based international representation and the excellent reports which resulted. Their recommendations have been followed by virtually all workers in human cytogenetics.

The question of an international registry of abnormal human karyotypes was first raised in 1966 because many people felt that unpublished data on individuals with abnormal chromosomes (and all laboratories of human cytogenetics have such data) could, if collected and pooled, be used in some worthwhile way. The topic was first considered in depth on April 7 and 8 of this year when a standing committee on human cytogenetics, set up after the 1971 international meeting held in Paris,

sponsored a workshop in Edinburgh on human cytogenetic registries. The meeting was attended by nine individuals who run such registries, by consultants and by members of the standing committee.

The workshop first considered cytogenetic registries generally. It was agreed that it was absolutely essential that the aims and goals of a cytogenetic registry be clearly defined and that the output of useful data from such a registry was dependent upon, and limited by, the methods of ascertainment employed in the registry and by the quality and uniformity of the information within it. Subsequent to discussion of the methods of intake into a registry, the question of patient confidentiality, the rigorous quality control needed for data in the registry and the necessity for the continued surveillance of patients for certain types of registries, the participants turned their attention to the question of an international registry of abnormal human karyotypes. After considering a variety of problems to which cytogenetic registries might address themselves, the participants decided that their solution would not be helped by an international registry.

Therefore, with one dissension (Dr D. S. Borgaonkar: see *Nature*, **253**, 591; 1975) they recommended that an international cytogenetic registry should not be set up at this time and that if, at some future date, an international registry should be considered of value, it should be established only after careful planning and under the guidance of an international advisory committee. They also recommended that the establishment of regional and national cytogenetic registries should be encouraged, as should cooperation between them and existing registries. They further advised, again with one dissension, that an international advisory committee on cytogenetic registries should be set up by the standing committee of the Paris conference and, lastly, that the standing committee should advise international organisations and appropriate national agencies of these developments and solicit their cooperation and support.

The workshop report was accepted by the standing committee who will publish it later this year as part of a supplement to the Paris Nomenclature. In the light of the recommendations the standing committee has already established an advisory committee on human cytogenetic registries under the chairmanship of Dr James R. Miller, Department of Medical Genetics, University of British Columbia, Vancouver.

At a time when few regional or national registries have yet shown their worth, it seems that the Edinburgh

workshop report reflects a very sensible decision not to support at present the establishment of an international cytogenetics registry which would, in all probability, turn into a cumbersome, expensive and unproductive way of disposing of unpublished data.

Label triangulation becomes feasible

from a Correspondent

A Brookhaven Symposium on neutron scattering in biological research was held on June 2-6 at the Brookhaven National Laboratory, New York.

INVESTIGATING the topography of ribosomal proteins by the chemical methods discussed recently in *Nature* (**254**, 555; 1975) can only lead to a qualitative model of a ribosome. The standard methods for structure determination are diffraction methods. At the symposium two groups showed independently that the application of the 'label triangulation method' to ribosomes has become an experimental reality. This method is in principle capable of giving the complete quaternary structure of the 54 proteins in a ribosome in a quantitative and unambiguous way. It is based on low-angle scattering in solution and was first proposed for X rays (Hoppe, *Israel J. Chem.*, **10**, 321; 1972) and shortly thereafter for neutrons (Engelman and Moore, *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1997; 1972).

Scattering in solutions can be treated like gas diffraction. Using the corresponding theory it can be shown that the linear combination of four scattering curves $u = a + b - c - d$ (scattering of solutions of equal amounts of a, the molecular complex to be studied; b, the molecular complex labelled with two additional scatterers A and B; c, the molecular complex labelled with A only; d, the molecular complex labelled with B) corresponds to a damped sine curve with a wavelength inversely proportional to the distance AB (see also Kratky *et al.*, *Mh. Chem.*, **76**, 281; 1946; **78**, 295; 1948). A systematic labelling is possible by disaggregation of the ribosome (respectively of the 30S or 50S subunit) and by reaggregation, replacing unlabelled proteins by labelled proteins. In the case of X rays labelling can be done by introducing heavy atoms; in case of neutrons by replacing deuterium (in a completely deuterated subunit) with hydrogen. A triangulation of the distances leads to the quaternary structure. Approximately 200 distances can be measured in the 30S subunit and 500 in the 50S subunit.

It is evident that not only a considerable amount of diffraction work but also a great amount of biochemical work is necessary for a triangulation.

The difference scattering curve $u=a+b-c-d$ can be measured in two different ways: one can prepare and measure four solutions of the derivatives (as proposed by Engelman and Moore, 1972) or one can prepare two mixed solutions, which deliver respectively $a+b$ and $c+d$. It was shown in the X-ray triangulation paper (Hoppe, 1972), that in the latter case all inter-particle effects (even if stemming from aggregations) will be cancelled (see also Hoppe, *J. molec. Biol.*, **78**, 581; 1973). This is a very unusual situation in solution scattering. It is easy to see how important—even essential—this cancelling is for the label distance measurement in such a giant assembly as the ribosome. The averaged label concentration in a 50S subunit is only 1%. One is therefore forced to work on highly concentrated solutions (11% of the 50S subunit and more than 20% of the 30S subunit in the later mentioned measurements). Moreover the additional determination of the shapes of the proteins in neutron work—which requires accurate scattering curves—would be entirely impossible.

The experimental results reported in Brookhaven have been achieved on the protein pairs S3–S7 (115 Å), S2–S5 (104 Å), S5–S8 (38 Å) in the 30S subunit (D. M. Engelman, P. B. Moore, B. P. Schoenborn, and collaborators) on the Brookhaven reactor and on L7/L12–L10 (100 Å), (W. Hoppe, H. G. Wittmann, H. L. Crespi, and collaborators) on the Grenoble reactor. In the latter case the two proteins L7/L12 are identical—except for a N-terminal group in L12. It has been deduced from other experiments that the pair L7/L12 should be a dimer. It can therefore be treated as a single protein. Both groups have used the mixture method; both labelled by replacement of hydrogen with deuterium (instead of *vice versa* as originally proposed in the neutron paper) in order to economise on deuterated material. The intensity differences are very small (with an order of magnitude of $\sim 1\%$) and could only be measured using low angle diffraction matrix instruments as first developed in Grenoble by K. Ibel.

Replacement of hydrogen by deuterium was first used in membrane work (Kirschner and Caspar, *Ann. N.Y. Acad. Sci.*, **195**, (ser. 5), 309) and it is therefore not surprising that much effort was reported in Brookhaven to use this type of isomorphous replacement not only for myelin membranes (D. Kirschner, University of Basle, and D. L. D. Caspar, Brandeis University) but also for other biological systems:

for example M. J. Yeager (Yale University) and M. Chabre, retina; V. Luzati (CNRS, Gif-sur-Yvette), lipoproteins; A. Miller (University of Oxford), collagen; E. M. Bradbury (Portsmouth Polytechnic), chromatin; J. Randall (University of Edinburgh), catalase. All work has been done by exchange of water or of hydrogen ions by respectively heavy water or deuterium ions. It is evident that the non-exchangeable replacement of core hydrogen by growth of microorganisms (as used in the triangulation work) will open up new possibilities. It is therefore of great practical importance that *E. coli* with nearly fully deuterated proteins can grow from hydrogenated substrates like glucose (P. Moore). Interesting applications of direct methods were reported, originating from earlier work of Hosemann and Bagchi (see Matter, *Direct Analysis of Diffraction*, Amsterdam, 1962). Of further interest is the fact that the first projects were aimed at the use of neutron spectroscopy in biological systems (J. White and W. L. Peticolas). A report on the Brookhaven Symposium would be incomplete without mention of the elegant work on protein crystal structure determination of haem proteins (B. P. Schoenborn, Brookhaven National Laboratory).

Tektites and their origins

from David W. Hughes

TEKTITES are small glassy objects which are found in certain localised areas on the Earth's surface. They are one of the enigmas of science, a cuckoo in a nest of meteorites.

There is still considerable doubt as to whether tektites are meteorites because they have never been seen to fall to ground. Meteorites are found randomly over the whole Earth whereas tektites only occur in certain areas, all at relatively low latitudes: the southern half of the Australian continent, Indomalaysia, the Ivory Coast in Africa, Czechoslovakia and North America (Texas, Georgia and Martha's Vineyard). They do not arrive at Earth continuously either: the separate arrival events all occurred during the last 34 Myr.

About 650,000 tektites had been discovered by 1960. The Czechoslovakian Moldavites are dark green whereas all the others are jet black, their surfaces usually having a dull lustre due to abrasion and weathering. The glass is isotropic and brittle with a refractive index between 1.48 and 1.52 and a density around 2.4 g cm^{-3} . Tektites have weights up to 300 g and shapes varying from spheroidal to pear-, lens- and disk-shaped. Many of them show ablation

features and contorted flow structures as if their surfaces had been heated while travelling at high speed through a gaseous atmosphere and subsequently quickly cooled. The chemical composition is that of a silicic igneous rock with high SiO_2 (70–80%) and moderate Al_2O_3 (11–15%), the composition being relatively independent of the formation with which the tektites are associated. There are, however, distinct minor variations between the different groups and also systematic minor variations across the individual strewn fields. Tektites also contain minute shred-like inclusion of pure silica glass (lechatelierite) which can only form at temperatures in excess of $1,710^\circ\text{C}$. Tektite composition is quite different from that of any known meteorite. The age of solidification of the primary object was found to be close to 400 Myr by using $^{87}\text{Rb}/^{87}\text{Sr}$ decay, and measuring the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio. The range of values obtained is much smaller than that found for terrestrial igneous rocks and there are only slight differences between specimens from different localities. By using the $^{40}\text{K}/^{40}\text{Ar}$ ratio, the age of solidification of the glass during ablation can be found. This is equivalent to the age of arrival on Earth and varies considerably between locations, being 0.61 Myr for Australites, 1.3 Myr for Ivory Coast tektites, 14.7 Myr from Moldavites and 34 Myr for American Bédiasites.

The origin of tektites is still unknown. Theories can be divided into two main categories: terrestrial and lunar. One of the favoured terrestrial theories is that tektites were formed by the fusion of terrestrial material during the impact of giant meteorites. These meteorites also produced large craters in the Earth's surface such as the Ries Kessel structure in West Germany, 250 km away from the Moldavite strewn field, and the Bosumtwi crater in Ghana. The potassium–argon ages of the tektites and of the cryptic glass from around these craters agree surprisingly closely. However, these impactite glasses found near the craters are markedly different from tektites, being slaggy and porous and having abundant inclusion of partly fused material; also the problems of moving the tektite a distance of 200–300 km against air resistance and accounting for the 10^3 difference in H_2O content between terrestrial rocks and tektites are not trivial. The fact that large impacts have occurred throughout the history of the Earth and not just in the last 34 Myr must also be explained.

Another terrestrial theory advocates a crypto-volcanic origin. McCall (*Meteorites and their Origins*, David and Charles; 1973) states that Reis Kessel and Bosumtwi might be extinct volcanoes and not impact craters, the



A hundred years ago

Mirage on Snowdon

ON Monday, July 12, I, with a party, ascended Snowdon. The atmosphere was clear until we had reached within half a mile of the summit, when a light cloud rising stealthily from amongst the southern peaks enveloped it. Drifting towards us, when very near, the cloud dropped over the eastern shoulder of the mountain just where it dips towards Capel Curig. As we stood watching, great was our surprise and delight as we beheld painted upon it, not the *arc-en-ciel* with which we are familiar, but a complete and brilliant prismatic circle, apparently about thirty feet in diameter, in the very centre of which we ourselves were depicted, the image being somewhat enlarged but clearly defined; as we arranged the party in groups, or bowed to each other, every form and movement was faithfully reproduced in the picture. It was now about 8 o'clock, with the sun nearly in a line with us. Our guide, who had made some hundreds of ascents, had never witnessed such a sight before.

H. J. WETENHALL

from *Nature*, 12, 292; August 12, 1875

tektites being produced by fusion between volcanic emanations and country rocks. A simpler theory postulates that tektites are shed melts derived from ablating meteorites. But tektites are acidic and all known meteorites are basic.

Lunar theories propose that the tektites are ejected from the Moon in a molten form during the formation of craters by meteorite impact and during periods of lunar volcanic activity. The composition of tektites is hard to account for as most of the material brought back from the Moon has been basaltic rather than acidic. The fact that there are only four age groups of tektites, although the hundreds of thousands of lunar craters were created at many different times, counts against the impact ejecta theory. Also impact craters mainly eject matter at low angles.

Lunar volcanic ejecta as a source of tektites is strongly advocated in the recent paper of Cameron and Lowrey in *The Moon* (12, 331; 1975). Reliance on a volcanic ejection process can explain the rarity of tektite falls on Earth and their composition. The velocity of escape they need to leave the Moon requires the volcanism to be of the explosive type which terrestrially is usually associated with acidic suites. (Acidic lavas, which have very high

viscosities of 10^{12} – 10^{13} poise, can sustain very high pressures before disruption. This leads to massive rock and lava ejection, as with Krakatoa which ejected 18 km^3 .) As the youngest tektite is only 0.6 Myr old, lunar volcanic activity must have been very recent—and the authors tie this in with observations of lunar transient phenomena—mild degassing observed from time to time around certain craters. The lack of H_2O in lunar samples is also echoed in tektites.

Tektites ejected vertically from the Moon at a speed of 3 km s^{-1} will only reach the Earth if they start from a small area on the eastern hemisphere within 6° of the lunar equator. The orbits are hyperbolic with respect to the Earth–Moon system so if the tektite misses the Earth on the first pass it goes into a heliocentric orbit. In searching for the exact point of origin Cameron and Lowrey regard 'lunar transient phenomena' sites within the specified boundaries as prime suspects, provided, however, that other evidence of volcanic activity can be found in their vicinity. The sites are all ray craters—Censorinus, Taruntius, Messier and Messier A (the old Pickering) and it is postulated that the rays are whitened exposed rocks which have had their dust covering blown off by gases emanating from the craters. Censorinus AB has a central mound with a funnel-shaped crater similar to those of terrestrial diatremes, fumaroles and explosion pits, all manifestations of volcanism. The hummocky bands in Messier, some of which have summit craters, are also pointers towards volcanism. Steep sloped ridges inside Taruntius indicate that volcanism could have post-dated the impact explosion.

The source of tektites must lie deep within the Moon because tektites differentiated in the last 10^9 years when the Moon's heat was confined to very deep regions. Cameron and Lowrey postulate that diatremes coming from these depths discharged their ejecta vertically; and that craters such as Messier were formed by impact and then suffered volcanism because the craters were formed on mare surfaces, the scenes of original volcanic flows.

So old lunar volcanoes, the present day sites of transient lunar phenomena, provided they are in the right area of the Moon, can eject tektites which will hit the Earth. But why have these only erupted in the last 34 Myr? Why are the tektites so tightly bunched in space that they only produce small strew fields on Earth? How does such pure differentiated material get produced on the Moon? Where are all the tektites that missed the Earth the first time, only to fall to Earth an indeterminate time later? Many questions remain unanswered; these enigmatic tektites

have been bothering scientists since the eighteenth century and I am sure will continue to do so long into the future.

Far infrared astronomy

from M. Rowan-Robinson

The first international conference devoted exclusively to far infrared astronomy was held at Cumberland Lodge, Windsor Great Park on July 9–11. It was organised by Queen Mary College, London, and sponsored by the Royal Astronomical Society.

THE far infrared is usually taken to mean the wavelength range $30 \mu\text{m}$ to 1 mm which, being almost inaccessible from the ground, is observed from aeroplanes, balloon platforms or (for the future) satellites. One of the most interesting discussions was of the relative merits of NASA's proposed Large Space Telescope (LST), a satellite-borne 60-cm telescope which will operate in the optical and ultraviolet as well as the infrared (plans were reported at the meeting by D. E. Kleinmann of Harvard), and balloon-borne telescopes working at an altitude above 30 km . One advantage of balloon-borne systems is that they are already in action. The University College, London group reported the latest results of their broad-band (40 – $300 \mu\text{m}$) survey of the galactic plane and maps of some individual sources, while G. G. Fazio of Harvard described $70 \mu\text{m}$ studies of several galactic HII regions and some external galaxies. K. Shivandan of Naval Research Laboratories, Washington, described multicolour photometry of the Orion nebula, and several groups described interferometer systems to be flown on aircraft or balloons giving detailed spectra of strong sources in the far infrared.

Another area of outstanding interest was the microwave background, with reports of Queen Mary College's balloon-borne interferometric observations in the range 0.7 – 2 mm and of Leeds's forthcoming experiment. Although the Berkeley group, who have recently successfully repeated the experiment, were not represented, much of the discussion centred on the conflicting atmospheric measurements and interpretation of the Queen Mary College and Berkeley groups. The issue remained unresolved, partly due to ignorance about the amount of ozone at altitudes above 30 km , and it is to be hoped that the Leeds experiment this summer will help resolve the issue. However both groups agree on the major issue, that the cosmic background spectrum turns over shortwards

of 1 mm as expected if it is the 2.7 K black-body relic of the fireball phase of a big bang Universe. The efforts of J. V. Narlikar (Tata Institute, Bombay) to explain this background as starlight thermalised by large graphite 'whiskers' ran into heavy criticism.

Ground-based astronomy had its moments too, with F. F. Gardner of CSIRO, Australia, L. T. Little of the University of Kent, and A. Gillespie of QMC reporting on molecular line studies (formaldehyde, H₂O and CO respectively) and P. E. Clegg reporting on Queen Mary College's 1 mm continuum studies of galactic sources, galaxies and QSOs.

The theoreticians produced a variety of models of dust-clouds and HII regions, disagreeing wildly over the composition, shape and size of the dust grains, ranging from ice and silicates to formaldehyde polymers, from spheres to whiskers and snowflakes, and from 0.1 μ m to 1 mm in size. Clearly much work remains for both observers and theoreticians in this exciting field.

Rock fabrics

from Robert W. Cahn

THE British Standard Conference is organised by a learned or professional society, lasts 2.5 days, has two parallel sessions, costs £25 in registration fees alone, involves a pricey conference dinner, is attended mostly by academics and industrial scientists whose institutions are still willing to pay up, and evokes a British Standard Response to the effect that, with luck, just one paper will be really interesting to the British Standard Conferee. I claim a little artistic licence—the British Standards Institution has not yet, so far as I know, laid down a standard for the BSC—but all seasoned scientists will recognise the gestalt.

Some weeks ago, in mid-May, I had the good fortune to take part in a very different kind of conference in the Geology Department at Imperial College, London. It lasted one day, cost £0.50 to attend, had a single session, and was organised entirely by a graduate student, K. McClay, on behalf of a group of graduate students and university staff (the Tectonic Studies Group); the audience consisted of a sprinkling of British and foreign academics and a lot of students, post- and undergraduate alike. The topic was "Fabrics and Textures in Rocks".

The term 'fabric' in a petrographic context denotes both preferred orientations in populations of crystal grains (what metallurgists term 'texture') and the shapes and mutual dispositions of matrix grains and subsidiary phases (what metallurgists term 'morphology').

Texture arises from mechanical deformation of rocks under pressure and resultant recrystallisation when the temperature is high enough. The processes involved must be related to those that generate deformation textures and annealing textures in metals; the Tectonics Group evidently recognise this, because the two opening speakers (I. L. Dillamore of the British Steel Corporation and R. W. Cahn of Sussex University) were invited to outline the present state of metallurgical knowledge on the two kinds of textures. The geologists present showed an impressive familiarity with the mathematical techniques used to interpret the genesis of deformation textures or the processes that arise during annealing of deformed metals. Thus G. Lister of the Geological Institute, Leiden, dealt with deformation textures in quartzite and S. White of Imperial College with recrystallisation mechanisms in the same rock, both drawing extensive metallurgical parallels.

Other contributors (E. Rutter and W. Shaw of Imperial College and R. G. C. Bathurst of Liverpool University) reviewed textures and fabrics of limestones, dolomites and marbles. D. J. Barber and H. R. Wenk (Essex University) applied electron microscopy to these rocks; this technique has only recently proved feasible for petrography, following the introduction of the ion-beam thinning technique, and it seems likely to prove as fruitful here as it has done in metallurgy.

The petrographer has the advantage over the metallurgist that he can examine his (optically anisotropic) minerals by transmitted polarised light, with the aid of a universal stage. Students of quartzite use this approach to determine a 'partial texture', that is, the distribution of the orientations of *c* axes both from one grain to another, and between different parts of the same grain: in this way, detailed textural and morphological information on a population of over 100 grains can be obtained within a day's work—information that would take a metallurgist, working with X rays and optical microscopy, or with electron microscopy, many weeks to assemble. Dr White in particular, using the *c*-axis technique, was able to draw on metallurgical experience to prove the close similarity of the processes that generate high-temperature textures in quartzite.

The remaining speakers, from Leeds and Leiden, dealt with more complex minerals, such as slates and peridotites. Here again, metallurgical processes such as strain-induced grain-boundary migration were identified.

One of the quartzite specialists spoke a few weeks later at a British Standard

Conference (good of its type!), devoted to textures in metals: to a witness who was able to compare the two occasions, it was plain that the geologists were much more ready to learn from metallurgical insights than metallurgists were willing to interest themselves in geologists' problems and techniques. This is a pity, because the two groups have much to contribute to each other.

Nuclear pores

from J. R. Tata

ONE of the many mysteries surrounding the organisation and function of the cell nucleus is how this organelle maintains concentrations of ions and small molecules (especially nucleotides) higher than those in the surrounding cytoplasm, while, at the same time allowing the large ribonucleoprotein precursors of polyribosomes to pass into the cytoplasm. The characteristics of the double nuclear envelope are such that some specialised site at the nuclear surface must regulate the nucleo-cytoplasmic transactions. A likely candidate for this function is the 'nuclear pore complex' (see Franke and Scheer in *The Cell Nucleus*, vol. 1, 219, 1974 for an excellent review). Although cytologists have for a long time known about the existence of pores on the surface of the nucleus, virtually nothing is known about their chemical nature and function.

Ultrastructurally, the size (diameter 600 ± 150 Å), but not the number, of nuclear pores is remarkably constant in all eukaryotic cells. Franke's group have done considerable work in defining optimal conditions for observing these structures by electron microscopy of intact cells and of isolated nuclei and nuclear membrane preparations. A well known ultrastructural feature of the nuclear pore is the 'annulus' of non-membranous material surrounding both the inner and outer rims of the pore (Callan and Tomlin, *Proc. R. Soc.*, **B137**, 367; 1950; Afzelius, *Expl Cell Res.*, **8**, 147; 1955). What is particularly interesting about the annulus is its characteristic eight-fold symmetry arising from eight 'annular granules' with diameters of 100–250 Å (Roberts and Northcote, *Nature*, **228**, 385; 1970; Franke and Scheer, *J. ultrastruct. Res.*, **30**, 288; 1970). The space within the pore is often seen to be filled with varying amounts of an 'amorphous' material as well as fibrils of about 50 Å diameter, themselves sometimes studied with smaller granules of 50–100 Å. Not surprisingly, several models for the structure of this complex ensemble have been proposed.

Blobel's group at the Rockefeller

University have undertaken to isolate and characterise the nuclear pore complex as a prerequisite for exploring its function. Recently, Aaronson and Blobel (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 1007; 1975) have succeeded in isolating the nuclear pore complex of rat liver nuclei and giving a preliminary account of the composition and structure of this isolated subnuclear fraction. The procedure involves, first, the preparation of nuclear envelope, not by a method based on treatment with high concentrations of Mg^{2+} as previously used in Blobel's laboratory (Monneron, Blobel and Palade, *J. Cell Biol.*, **55**, 104; 1972), but one based on the use of DNase I (Kay, Fraser and Johnston, *Eur. J. Biochem.*, **30**, 145; 1972). The isolated nuclear membranes are then treated with detergent (Triton X-100) to solubilise phospholipid and remove the membraneous components; any residual chromatin is removed by precipitation of the nuclear pore complex with 0.3 M $MgCl_2$.

When examined in the electron microscope, Aaronson and Blobel's isolated nuclear pores were found to be associated with stretches of the peripheral lamina of the nucleus. The latter structure, which is distinct from the inner nuclear envelope, has been previously described in intact nuclei from other sources but not from rat liver (Fawcett, *Am. J. Anat.*, **119**, 129; 1966; Kalifat, Bouteille and Delarme, *J. Microsc.*, **6**, 1019; 1967). The lamina in the isolated preparation is 150 Å thick and connected to well preserved pores of diameter 700–900 Å whose lateral profile is 650 Å in diameter and 'goblet-shaped'. An interesting feature of these isolated structures is the absence of any membraneous material, which confirms an earlier suggestion by Aaronson and Blobel (*J. Cell Biol.*, **62**, 746; 1973), based on the preparation of 'nuclear ghosts', that the membrane of the double nuclear envelope is not essential for imposing or maintaining the structure of the pore complex. As regards its composition, the isolated rat liver nuclear pore complex is almost 94% protein with traces of RNA and DNA but no detectable phospholipid. The sugar content has not been determined and it would be interesting to know whether any glycoproteins are present. Electrophoresis on sodium dodecyl sulphate-polyacrylamide gels resolved the proteins of the isolated nuclear pore complex associated with the lamina into three major components of molecular weights 66,000, 68,000 and 69,000 with a minor component of much higher molecular weight. This simplicity of protein composition is, indeed, surprising in view of the complex structural elements of the nuclear pore.

What could be the functions of the nuclear pore complex in the intact cell? That the number of pores per nucleus is not constant, but is directly related to the rate of protein synthesis or ribosome accumulation (as during amphibian oogenesis), suggests a role in the nucleo-cytoplasmic transport of ribonucleoprotein particles. Isolated nuclear pore complexes do not offer any advantage in studying such a transport function, which can best be tackled by a combination of indirect methods of cell biology and cytology. But the advance made by Aaronson and Blobel offers the possibility of directly testing the role of the pore complex in carrying out terminal modification step(s) in the biogenesis of messenger and ribosomal RNA and the assembly of corresponding ribonucleoprotein particles. Thus, it would be interesting to see if the isolated nuclear pore complex has enzyme(s) for specific cleavage, polyadenylation or methylation of RNA, or whether it could somehow facilitate the terminal addition of protein(s) to 'immature' ribonucleoprotein particles just prior to their release into the cytoplasm. However, the extremely simple protein composition of the isolated nuclear pore complexes makes it likely that they retain few, if any, of such activities as they may possess in the intact cell.

Links between core and plates

from Peter J. Smith

IN a series of articles published about a decade ago, Wilson and his colleagues (see, for example, Wilson and Haggerty, *Endeavour*, **25**, 104; 1966) reported that they had established a statistical correlation between magnetic polarity and the degree of oxidation of the iron-titanium oxides in a wide variety of basalts. Specifically, they showed that the higher the average oxidation state of the oxides within a basalt, the greater was the chance of the rock having reverse magnetisation. This result was, and remains, a mystery. The magnetic polarity of a rock depends on the polarity of the geomagnetic field at the time the rock formed; and the polarity and polarity changes of the field are presumably governed by processes in the Earth's core. Lava flows, on the other hand, originate in the upper mantle. The Wilson correlation thus seemed to imply an unlikely connection between core and upper mantle processes.

Since then, the so-called 'oxidation state-polarity paradox' has been largely ignored, partly because some workers have disputed its validity and partly because even those who accept the cor-

relation have been unable to decide whether or not it is significant enough to follow up. But the question of links between core and mantle was later revived by Hide and Malin (*Nature*, **225**, 605; 1970) who found a significant correlation between the Earth's gravitational field and the non-dipole geomagnetic field. This they attributed to undulations of the core-mantle interface, which would presumably influence, and be influenced by, both core and mantle motions. It also seemed to bear out in part Hide's (*Science*, **157**, 55; 1967) earlier prediction that "it would not be surprising to find that 'reversals' are correlated to some extent with other phenomena that may be affected by motions in the mantle, such as tectonic activity (mountain building, ocean-floor spreading, continental drift, and so forth) . . .".

The conceptual difficulty raised by the Wilson correlation of the mid-1960s had thus given way by 1970 to one possible physical model whereby core-mantle interaction could be achieved; since then other connections between mantle and core processes have been proposed. Won and Kuo (*J. Geophys. Res.*, **78**, 905; 1973), for example, have suggested that great earthquakes could cause the solid inner core to oscillate. Any such oscillation could be expected to influence fluid motions in the outer core and hence affect the apparently delicate mechanism of field reversal. Closer to the Earth's surface, on the other hand, the exciting earthquakes are intimately related to plate tectonic processes. The Won-Kuo hypothesis thus implies that correlations between geomagnetic field behaviour and events in the crust and upper mantle are not only possible but probable.

The near-surface phenomena envisaged here are altogether more conspicuous—Vogt (*Earth planet. Sci. Lett.*, **25**, 313; 1975) calls them 'first order' events—than the previous polarity-oxidation and gravity-magnetic field correlations. But the motion of lithospheric plates is also complex, making it difficult to define a general plate tectonic 'index' with which to compare geomagnetic behaviour. Any attempt at a general worldwide comparison is thus out of the question for the time being. As an alternative, however, Vogt has examined the four principal plates for particular examples of correlations between plate tectonic activity and geomagnetic phenomena, and has discovered enough of them to suggest that core-upper mantle coupling is real.

The first comes from the Hawaiian-Emperor chain of volcanic islands in the Pacific—a chain which comprises two more or less linear sections joining at an angle. Because these islands

increase in age from one end of the chain to the other, they have been attributed to the motion of the Pacific plate over a stationary hot spot, possibly related to a mantle plume (although other explanations are possible). The bend in the chain is then thought to be due to a sudden change in the direction of Pacific motion which can be dated from the monotonous age pattern of the islands at 42–45 Myr. But the geomagnetic reversal frequency also changed significantly at that time. Thus within the errors of dating, the only conspicuous changes in Pacific plate motion and reversal frequency during the last 70–80 million years coincide. Moreover, other tectonic changes took place at the same time in other parts of the world. In the Reykjanes ridge area south of Iceland, for example, the 45 Myr isochron marks the largest change in the direction of motion between Greenland and Europe, a change which gave rise to the generation of numerous small transform faults.

The second magnetic event examined by Vogt was the ending 70–77 Myr ago of the Mercanton interval, a 30–40 Myr period during which there were few or no reversals. At this time, seafloor spreading first began south of the Rockall Bank in the northeast Atlantic and the Meteor and Corner Rise seamount groups were formed. In addition, there was a major change in the trend of the fracture zones in the central North Atlantic, a reflection of what appears to be the most prominent change in spreading direction between North America and Africa during the whole 180 Myr existence of this ocean basin. Other major plate tectonic changes also took place about 115 Myr ago when the Mercanton interval began and the earlier Keathley sequence of reversals (150–115 Myr) ended.

But if these correlations are manifestations of core-mantle coupling, the problem of precisely how the coupling occurs remains. Vogt clearly favours a return to the idea of mantle-wide convection, the most obvious and traditional physical link between the core-mantle boundary and the near-surface processes. In this case, the geometry of the convection would change from time to time, thereby directly influencing the plate motions above and at the same time changing the boundary conditions at the core-mantle interface and hence the reversal frequency within the core. Whole-mantle convection has not received much support in recent years. Since Wilson's basalt correlations (which are not mentioned by Vogt) were discovered, however, an alternative form of mantle-wide motion has been proposed in the form of mantle plumes. These would seem to offer a connection between core and

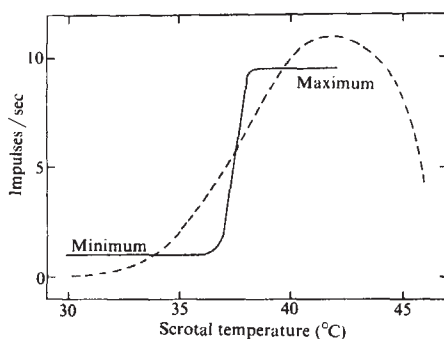
plates which is both more direct and perhaps more attractive to today's earth scientists.

Convergence of information in thermal pathways

from Shin-Ho Chung

NEUROPHYSIOLOGICAL studies of different sensory systems have shown that incoming information undergoes drastic modification as it proceeds along the pathways of the nervous system, the degree of modification increasing as messages proceed centrally. Such hierarchical organisation is a general principle upon which all the nervous system is constructed, and understanding the transformation of information at each level has been the dominant preoccupation of sensory neurophysiologists over the past two decades. Two recent articles by Hellon, Hensel and Schäfer (*J. Physiol., Lond.*, **248**, 349–357; 1975) and Hellon and Mitchell (*ibid.*, 359–376) describe the successive steps taken by the nervous system in processing the information generated by thermoreceptors.

Primary sensory fibres arising from 'cold' and 'warm' receptors in the rat scrotum show action spectra resembling bell-shaped curves when frequency of impulses is plotted against scrotal temperature. The size of each receptive field is about 1 mm². These inputs are integrated by cells in the spinal cord; however, cells in the cord no longer have spot-like receptive fields but are sensitive to temperature changes over the entire extent of the scrotum. Alteration of the response pattern is most clearly seen in the behaviour of cells in the thalamus. Unlike the bell-shaped action spectra of primary afferents, thalamic cells exhibit an on-off pattern of firing (see figure). A cell remains silent until a critical temperature is sensed, after which it fires at its maximal discharge frequency.



Action spectra of 'warm' receptors in the rat scrotum (broken line) and of thalamic neurones (continuous line). From Hellon and Mitchell, *J. Physiol., Lond.*, **248**, 349–357; 1975.

Different cells in the thalamus have different critical temperatures (Hellon and Misra, *J. Physiol., Lond.*, **232**, 389; 1973). Thus, at the expense of spatial discrimination, activities of primary afferents are integrated by the central nervous system and, as thermal assault on the testes intensifies, more neurones are recruited in their defence. This is a sensible physiological arrangement, for the ability to discriminate which side of the scrotum is warmer is unlikely to serve any useful biological purpose.

Language understanding by computer

from Arnold G. Smith

A conference at MIT on June 10–13 entitled "Theoretical Issues in Natural Language Processing" was aimed at scientists working on computer models of language either in order to study the human mind, or with the more pragmatic goal of "teaching computers to speak English".

ATTEMPTS to create computer systems which understand English raise issues which reach deeply into linguistics, computer science, psychology and even philosophy. Earlier work in this field led to the realisation that a reasonable model of language comprehension would require the active use of a potentially vast amount of 'common sense'. Common sense is called upon continually though often unconsciously in making sense of what we hear, but how does one design a computer program to function analogously, organising a large corpus of knowledge so as to have the appropriate facts available at the appropriate moment? Marvin Minsky's concept of frames is the currently fashionable approach to answering this question, although it generated a great deal of controversy at the meeting.

Minsky (from MIT) was there to explain his ideas. He uses the term 'frame' basically in the sense of frame of reference, in the light of which one makes sense of a sentence, a story or a scene. An intelligent person presumably has a very large number of such frames stored away in a richly interconnected structure. At any point one of these frames is in charge of interpreting and interacting with the current environment. It has its own procedures which look for evidence to confirm its relevance to the situation at hand and, when something turns up which is inconsistent with or beyond the scope of the current frame, it has

links to likely alternative frames which will have a good chance of making sense of the input. Each frame has slots for the kinds of information it expects to find and, until these slots are filled, they are likely to have default assignments. My frame for bedrooms, which will be evoked if you start to tell me what happened in your bedroom, has defaults for things like the size of the room, and the style of the bed. In the absence of specific information, I will simply assume the presence of a normal bed to supply the default. If you then say that ten people slept in your bed last night, I will expect them to have been very crowded. On the other hand, an earlier description of your particularly huge bed would have displaced the default and so have led to different inferences. As long as a particular frame is in charge, it coordinates the information that comes in, making inferences to connect what might otherwise look like unconnected bits of evidence, and calling on other frames with narrower expertise to work with details of the situation. In this way, frames supply an interpretive context and constitute their owner's model of the world.

Some critics regard the frame theory as a re-statement of ideas long current in philosophy and psychology. In fact Minsky explicitly disavows claims of formal precision for his theory, but it is oriented specifically towards computer models of cognition. Terry Winograd of Stanford pointed out various ways in which frames were suggestive of, but not merely equivalent to, a variety of other paradigms from artificial intelligence—scripts, patterns, decision trees, semantic nets, actors, and production systems. He suggested that frames share important characteristics with each of these, but that you miss part of the point of frames by limiting your perspective to any one. I see the frames concept not as a unified theory but as a vantage point for discussing the shortcomings of present systems and pointing to some of the properties that better cognitive models must have.

A workshop session on methodological issues considered the difficult problem of evaluating and comparing the contributions of different projects in the field. Two factors seem to exacerbate the situation. One is the notorious lack of perspicuity of large computer programs. The other is the fact that language is so inextricably involved with everything we do that it is hard to gain the perspective needed to see how a particular piece of work fits into the whole domain. W. A. Woods, of Bolt, Beranek and Newman, presented a particularly well-reasoned paper on this subject. He argued,

among other things, the need for cognitively efficient representations—models whose explicit structure can act as a guide to understanding the theory they incorporate.

There was somewhat less than one might have expected in the way of new results reported at the meeting. Several people were in the process of working out some of the implications of their version of the frames idea. Roger Schank and his colleagues at Yale, for example, described initial work with a program which knows what to expect when it hears a story about someone going to a restaurant. A few sentences relating John's experience eating a lobster dinner will trigger off a variety of inferences about who cooked the lobster, how it arrived at John's table, that if he gave the waiter a large tip it was probably because he enjoyed the lobster, and so on. And Sheldon Klein, of the University of Wisconsin, passed out copies of a seven page murder story generated by his computer in nineteen seconds! But there was more emphasis on what people had been thinking about methods and paradigms than on what they had learned about language.

Motion of muscle proteins

from a Correspondent

THE dynamic behaviour of proteins—their rotation and internal flexibility—are important for many aspects of their biological function. This is particularly true in the actomyosin system of muscle, where these phenomena are intimately related to the mechanism of contraction.

In such a situation, it is obviously necessary to be able to study the behaviour of individual components of the system, and in practice this means they must be labelled in some way. Two such labelling techniques have been widely used over the last few years. In the first, the protein is labelled with a strongly fluorescent group; the time course of the decay of the anisotropy of the fluorescence after excitation with a short flash of polarised light can then yield a value for the rotational correlation time, τ_c , of the label. The second approach, known as spin-labelling, involves the attachment of a stable nitroxide free radical to the protein. The value of τ_c is obtained by careful analysis of the shape of the electron paramagnetic resonance (EPR) spectrum of the nitroxide. Both of these methods are at their best in the study of relatively rapid motions—those with correlation times shorter than a few times 10^{-7} s.

Recently a new EPR technique has been developed (Hyde and Dalton, *Chem. Phys. Lett.*, **16**, 568; 1972; Hyde and Thomas, *Ann. N.Y. Acad. Sci.*, **222**, 680;

1973) which promises to extend the application of the spin-labelling method into the time range 10^{-7} – 10^{-3} s. This technique, known as saturation transfer EPR spectroscopy, depends upon the use of partially saturating microwave fields and out-of-phase detection to obtain a spectrum whose shape depends critically on the rate of rotational diffusion. The theory of the method is discussed by Thomas and McConnell (*Chem. Phys. Lett.*, **25**, 470; 1974); the maximum sensitivity to motion occurs when $\tau_c \sim 1/\omega_m \sim T_1$ ($\sim 10^{-5}$ s), where ω_m is the modulation frequency and T_1 the electron spin-lattice relaxation time.

This technique has now been applied to myosin and its interaction with actin (Thomas, Seidel, Hyde and Gergely, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 1729, 1975). The spin label was attached to a thiol in the globular 'head' (subfragment 1) of the myosin molecule. The correlation time of the isolated subfragment 1 was 1.8×10^{-7} s, and for myosin this increased to 3×10^{-7} s. Thus there is only about a 40% decrease in τ_c when subfragment 1 is cleaved from myosin, although the molecular weight decreases by a factor of more than four. There must therefore be considerable internal flexibility in the myosin molecule; the same conclusion was drawn earlier from closely analogous fluorescence experiments by Mendelson *et al.* (*Biochemistry*, **12**, 2250, 1973). The saturation transfer EPR technique can also be applied to supramolecular complexes of myosin, which are essentially beyond the range of previous methods. Aggregation of myosin into filaments led to a ten-fold increase in correlation time, indicating that there was still considerable flexibility of the 'heads' of the myosin molecules. In contrast, addition of actin to either subfragment 1 or myosin led to a very substantial immobilisation of the label, giving a correlation time of 10^{-4} s. This indicates that the globular 'head' of myosin is essentially irrotationally bound to actin.

The saturation transfer EPR method shares two problems with the other labelling methods. Motion of the label relative to the protein to which it is attached will obviously lead to erroneously short values of τ_c for the protein. Though this does not seem to happen in the case of subfragment 1, it may limit the application of these methods to some systems. In addition, for a non-spherical protein, rotational motion cannot strictly be described by a single correlation time, and the value obtained will depend on the orientation of the label relative to the hydrodynamic axes of the protein. But notwithstanding the difficulties in precise quantitation, it is clear that this new method is a valuable addition to the armoury of the physical biochemist interested in supramolecular structure.

review article

The origin of nuclei and of eukaryotic cells

T. Cavalier-Smith*

A new theory not involving symbiosis is proposed for the origin of eukaryotic cells. It explains how the evolution of phagocytosis by a wall-free blue-green alga would have created selection pressures leading directly to the formation of all characteristic eukaryote organelles and cell properties including mitosis, meiosis and sex.

THE problem of the evolutionary origin of eukaryotes has been a major one in biology ever since the fundamental distinction between prokaryotic and eukaryotic cells¹⁻³ became clear. It is generally accepted that eukaryotes evolved from prokaryotes, but how this happened is unknown⁴⁻⁵. Certain similarities between mitochondria and bacteria and between plastids and blue-green algae have led to the recent revival⁶⁻⁸ of old theories⁷⁻¹¹ suggesting that these eukaryote organelles are derived from intracellular symbiotic prokaryotes and also to the suggestion^{7,12,13} that microtubules, centrioles and flagella are similarly derived. This "serial endosymbiosis theory of the origin of eukaryotes", which supposes that eukaryotes evolved as a result of the symbiosis of from three to six^{7,14-16} genetically different prokaryotes, has received more support²⁻¹⁹ than the alternative theory that they evolved from a single prokaryote species by intracellular differentiation²⁰⁻²².

My strongest criticism of the symbiosis theory is that it fails to explain how the eukaryote condition itself (that is, the nucleus) evolved²³. Most proponents of the symbiosis theory⁵⁻¹⁹ do not seriously discuss the origin of the nucleus, but assume it to have evolved gradually from a prokaryote nucleoid.

Eukaryote nuclei differ in at least three fundamental ways from prokaryote nucleoids. They are surrounded by a double-membraned envelope bearing characteristic pores; they contain several non-identical chromosomes which are linear and not circular; segregation occurs by mitosis which always involves spindle microtubules. Here I show how these differences could have arisen and argue that the same selective forces would also have led to the formation of mitochondria, plastids and other characteristic eukaryote organelles and properties.

Like Stanier⁵ I consider the evolution of endocytosis (phagocytosis and pinocytosis) to be of key importance in eukaryote evolution. But this is not because it enabled them to harbour endosymbionts. (I believe endosymbiosis to be one of many secondary and almost inevitable consequences of phagocytosis, but not the cause of the eukaryote condition.) It is because phagocytosis provided not only the selective pressure but also the physical mechanism (membrane budding and fusion) for cell compartmentation by intracellular membranes. Cell compartmentation explains not only the origins of mitochondria, plastids and nuclei, but also their characteristic properties more simply than does the symbiosis theory.

I assume that the ancestor of all eukaryotes was a single-celled, facultatively phototrophic, blue-green alga, unable to fix nitrogen but possessing oxygen-evolving photosynthesis and oxygen-using respiration based on cytochromes and other electron transport molecules borne on intracellular thylakoid

membranes (Fig. 1a). The first step leading to eukaryotes must have been the loss of the cell wall by such an alga living in a shallow bacteria- and detritus-rich benthic environment (Fig. 1b). Cell wall degrading enzymes like those abundantly secreted by soil myxobacteria³ may initially have produced a blue-green algal "L-form" which subsequently became a stable L-form. Whatever the mechanism, wall loss was essential for phagocytosis and explains the complete absence of peptidoglycan cell walls in eukaryotes.

Many blue-green algae (in contrast to bacteria, whose minute size would make the evolution of phagocytosis difficult) have cells as large as those of unicellular eukaryote algae^{3,25}, so size would be no barrier to the acquisition of phagocytosis—they would already be large enough to engulf bacteria. A strong selective force would favour any blue-green algal L-form able to develop phagocytosis and so become a "pre-alga". A phagocytic pre-alga could photosynthesise by day or during the Arctic summer, and phagocytose by night or during the Arctic winter (or in any dark environment). This versatility would give it a clear advantage over other blue-green algae (mostly obligate phototrophs) as well as over bacteria since it would seldom lack food.

Evolution of phagocytosis

I suggest that there is a fundamental similarity between the mechanism of plasma membrane budding to form a phagosome and the mechanism of eukaryote cell cleavage during cytokinesis. Both involve invagination, breakage and resealing of membranes. Clearly they differ in the timing and location of these processes. But I think these reflect differences in control rather than in the basic mechanism, and that the blue-green alga evolved cleavage first and endocytosis evolved subsequently from it.

Phagocytosis, like division, reduces the surface area of the plasma membrane but, unlike division, creates separate intracellular phagosome membranes. Extensive endocytosis is therefore not possible unless the phagosome membrane can refuse with the plasma membrane after absorption of its contents. Thus phagocytosis could not evolve in the absence of this reverse process (exocytosis—frequently the basis of secretion in eukaryotes). Since, in growing (but not in non-growing) cells, limited exocytosis is possible in the absence of endocytosis, exocytosis probably evolved before endocytosis (either as a mechanism of membrane growth additional to the insertion of individual molecules into an existing membrane, or more probably for secretion of extracellular digestive enzymes). Any blue-green alga possessing both cleavage and exocytosis would be preadapted for the evolution of phagocytosis. Figure 1 shows a possible evolutionary sequence.

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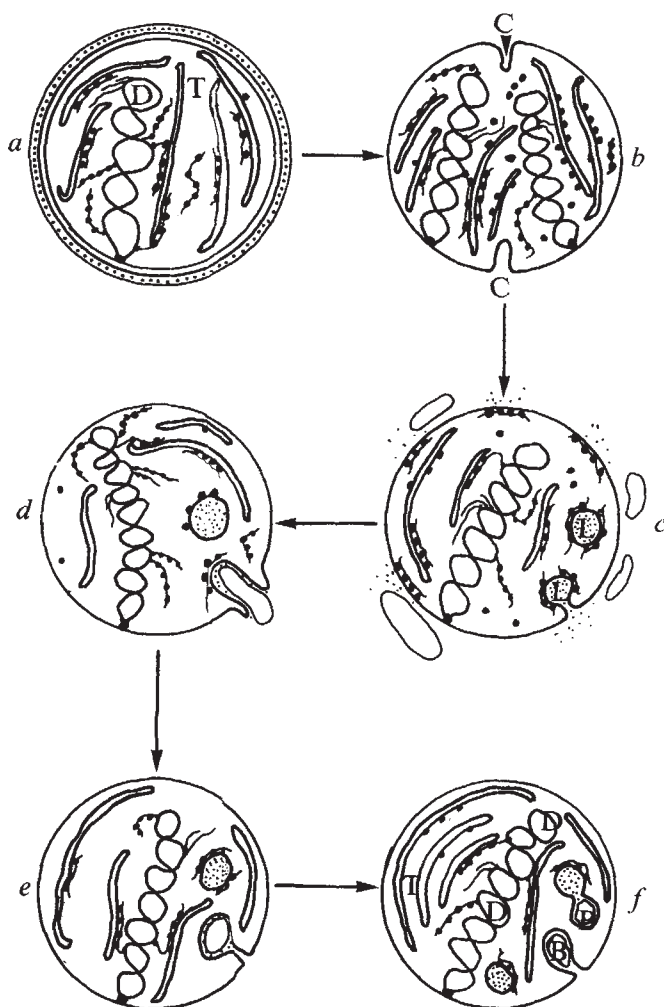


Fig. 1 The evolution of phagocytosis. A blue-green alga loses its cell wall (*a*) and evolves an actomyosin dependent cell cleavage mechanism (*b*). The resulting L-form develops extracellular digestion (*c*) by direct secretion of digestive enzymes across the plasma membrane and/or by exocytosis by protolysosomes (L) derived from thylakoids specialising in the intracellular storage of digestive enzymes. Efficiency is increased by partially (*d*) or completely (*e*) surrounding the prey before liberating the enzymes. Finally in the fully phagocytic "pre-alga" only the plasma membrane engulfs the bacterium (B) and the protolysosome fuses with the resulting phagosome. DNA (D), with its attached polysomes, and most thylakoids (T) remain unchanged.

I suggest that not only exocytosis, endocytosis and cleavage but all cases of controlled membrane budding and fusion in eukaryotes (for example, budding of smooth vesicles from rough endoplasmic reticulum or Golgi apparatus, or the fusion of transmitter vesicles with presynaptic membranes) have a common basic mechanism, which I call cytos. Since much of eukaryote cell evolution can be understood in terms of a diversification in the uses of and increased control over the timing and positioning of cytos it is a pity that so little is known of its mechanism. Conceivably membranes containing polyunsaturated fatty acids (found in blue-green algae and in eukaryotes but not in bacteria³) were a prerequisite. Although phospholipid membranes have a natural tendency to bud and fuse I suggest that cytos also universally involves a calcium-activated contractile actin-myosin-like microfilament system²⁶. The simplest explanation for the universality of actomyosin in eukaryotes² is that it was the essential molecular innovation which made the origin and evolution of eukaryotes possible, and that it originated when our blue-green algal ancestor lost its cell wall and the prokaryote type of cell division by localised growth of a semi-rigid membrane was

superseded by actin-myosin mediated cleavage by furrowing, that is by cytos.

Subsequently, modifications led to exocytosis and then phagocytosis. Then selection for greater phagocytic efficiency would occur. Cells would be selected for greater size and for the ability to phagocytose over their whole surface. The actomyosin system would diversify independently to produce amoeboid movement—greatly increasing predation efficiency—and cytoplasmic streaming and organelle movement—speeding up contact between phagosomes and protolysosomes.

Origin of the spindle

Such a highly mobile cell surface would interfere with chromosome segregation (dependent in prokaryotes on attachment to a stable semi-rigid membrane²). Endocytosis of the chromosome attachment site would be especially serious, so there would be strong selection for a new rigid non-membrane segregation mechanism—the microtubule. Initially microtubules joined the two membrane attachment sites (Fig. 2*a*) and pushed them apart as they grew (like pole-to-pole spindle microtubules, the only universal components of modern spindles^{24,29}; or like the reverse of sex pilus retraction (microtubules and sex pili are both tubular and interact with chromosome attachment sites—are they related or just functionally similar?)). This ensured that one chromosome ended up on each side of the cleavage furrow (a mechanism to ensure that cleavage was at the equator was also essential).

Efficient segregation by microtubules ended the selective advantage of chromosome attachment to the plasma membrane; the attachment site would soon be endocytosed and thereafter remain inside the cell as a protonuclear envelope (Fig. 2*b*), making the entire cell surface available for phagocytosis. The origin of mitosis was not a consequence, as commonly supposed, of the greater size or greater number of eukaryote chromosomes but was instead the essential prerequisite for these changes.

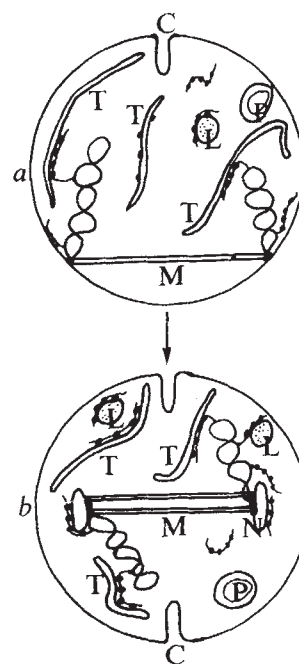


Fig. 2 The evolution of spindle microtubules (M) in the amoeboid pre-alga, as a device to push the two circular daughter chromosomes (here shown twisted into supercoils) apart to opposite poles of the cell to ensure that one is present in each daughter cell produced by the cleavage furrow (C). Initially chromosomes were attached to the cell membrane (*a*) but later (*b*) the attachment sites were endocytosed to become protonuclear envelope, N. Phagosomes (P), protolysosomes (L) and thylakoids (T) are also shown.

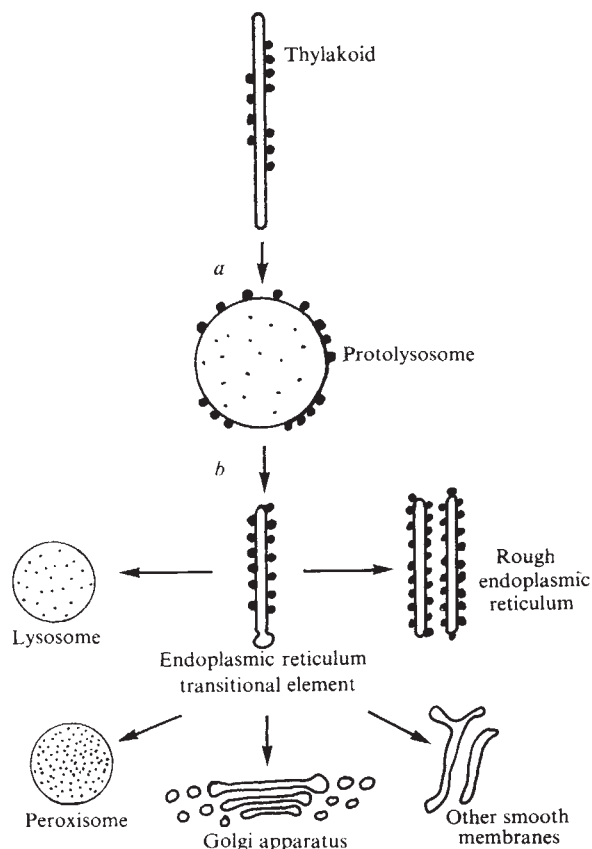


Fig. 3 Diversification of the (ribosome-covered) protolysosome (a) (originally derived from a ribosome-bearing thylakoid). The evolution of membrane budding by cytolysis allowed differentiation and specialisation between the "rough" ribosome-bearing membranes and their various smooth-membraned products (b).

Cytosis and origin of sex

Cytosis also provided the mechanism for cell fusion, which must have evolved in a wall-free cell, and initially was probably poorly controlled and more frequent than today. Its initial selective advantage would be greatest in times of starvation (starvation still triggers sexual differentiation in many algae and fungi) when fused cells would have twice the internal food supply of unfused competitors. I suggest that it originated in this way in the amoeboid pre-alga before even the development of the nuclear envelope; many properties of eukaryote chromosomes are best explained by the simultaneous evolution of mitosis and meiosis.

With only one circular chromosome per cell meiosis was unnecessary; the primitive mitosis described above would ensure segregation. Recombination was easy in the absence of a nuclear envelope. Assuming reciprocal and random recombination (as in the *rec* system of *Escherichia coli*), an even number of crossovers would produce two recombined daughters but an odd number would produce double length circular DNA. This increase in genome size and redundancy would immediately provide raw material for rapid evolution of new functions and create multiple replicon origins. The random release of spare copies of genes from stabilising selection pressure would break up operons. But an indefinite increase in DNA would be disadvantageous (and double-sized DNA molecules would often be broken during segregation because of their two membrane-microtubule attachment sites), and so selected against—most simply by the chromosomes becoming linear by the mechanism I previously suggested³⁰. With linear chromosomes odd as well as even numbers of crossovers will give normal reciprocal recombinants thus terminating the explosive burst of new genome creation.

Selection for increased recombination efficiency would lead to chromosome pairing mediated by a synaptonemal complex. Efficient pairing would remove the necessity for having all the DNA in one molecule (previously necessary to prevent aneuploidy). Indeed, positive selection for chromosome fragmentation by the mechanism previously suggested³⁰ is likely because this will give increased recombination by independent assortment of non-homologous chromosomes. Thus the most distinctive features of eukaryote chromosomes, mitosis, meiosis and sex probably all evolved in a very short space of time, during the earliest stages of eukaryote evolution as a direct consequence of the evolution of cytolysis. Chloroplast fusion³¹ was probably a late development.

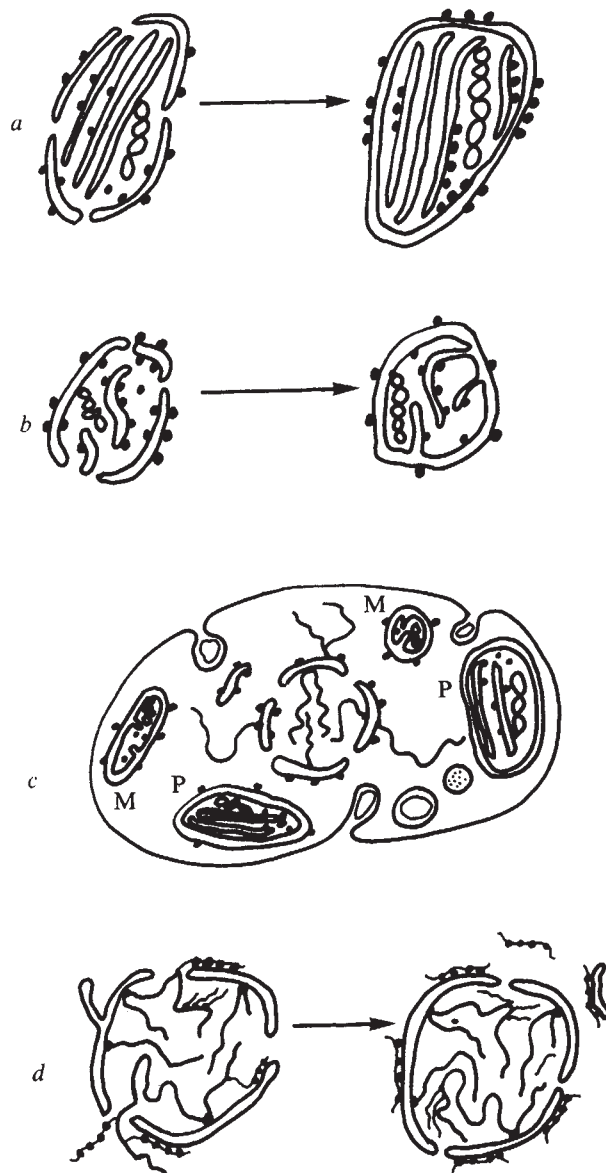


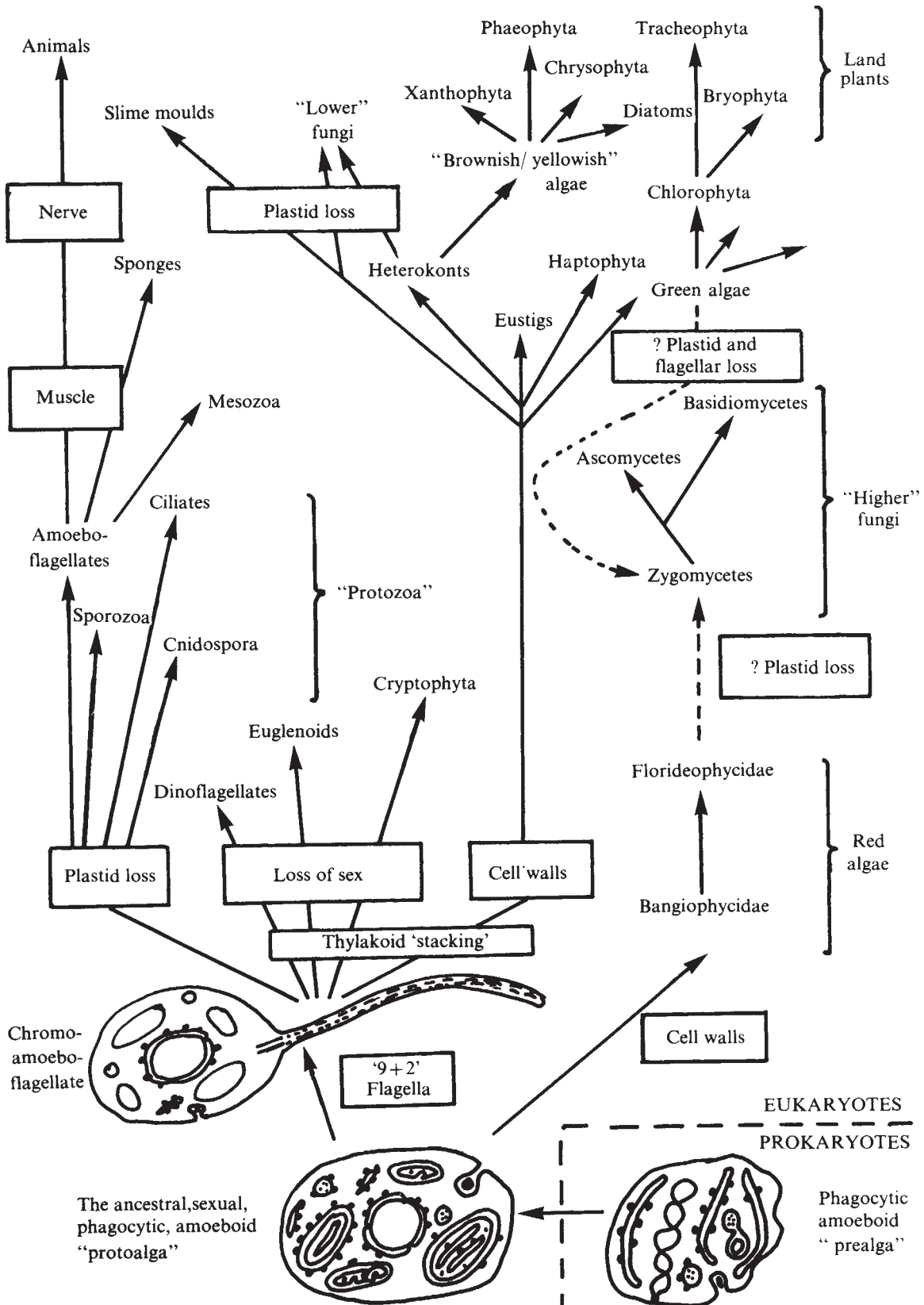
Fig. 4 The evolution of plastids, mitochondria and nuclei by fusion of DNA-associated thylakoids or endoplasmic reticulum cisternae. a, Plastids resulted from association of photosynthetic and plasmid-associated thylakoids to form a compartment containing DNA, ribosomes and Calvin cycle enzymes. b, Mitochondria resulted from fusion of plasmid-associated respiratory thylakoids to form a compartment containing Krebs cycle and fatty acid metabolising enzymes. c, Cell at intermediate stage with three distinct compartments: mitochondria (M), plastids (P) and nucleocytoplasm, whose protonuclear envelope (N) with six linear chromosomes will eventually fuse (d) to form a nuclear envelope separating the cytoplasm with protein synthesising enzymes from the nucleus with DNA and RNA synthesising and processing enzymes.

Cell compartmentation and organelle origins

Increase in cell size and in diversity of cell components in response to the selection pressures described above would dilute each component and lower the efficiency of reactions. This could be prevented only by vastly increasing the amounts

made and the total concentration of materials or by dividing the cell into compartments each specialising in different functions. Cells adopting the latter course would have a tremendous selective advantage. The evolution of cytosol not only provides the selective pressure but also the mechanism

Fig. 5 A possible pattern of eukaryote diversification following the formation of the first true eukaryote (red algal-like) "proto-alga".



for compartmentation.

The early ribosome-covered protolysosomes (Fig. 1) were already separate compartments (because of the need to secrete, and protect the cell from, their hydrolases) and could very simply have differentiated into rough and smooth endoplasmic reticulum, lysosomes, peroxisomes and Golgi apparatus as shown in Fig. 3; they and the original thylakoids could have formed the plastid, mitochondrial and nuclear envelopes by fusion as shown in Fig. 4.

Initially ribosomes, DNA, RNA and nucleic acid polymerases were divided into 3 compartments, Fig. 4c. The nucleoid in the cytoplasmic compartment was segregated by microtubules and its DNA became linear and fragmented as suggested above. Chloroplast and mitochondrial plasmids were present always in large enough numbers per cell not to require a special segregation mechanism, and so (generally) remained circular because with multiple copies the selection pressure for linearity was much less. In both plastids and mitochondria, DNA, messenger RNA and ribosomes are in the same compartment (as in bacteria) so would be expected to retain many prokaryotic properties. By contrast nuclear DNA and RNA became segregated from cytoplasmic RNA and ribosomes (Fig. 4d) so ribosomes could no longer start translating messenger while it was still being made and new mechanisms were required for the transport of messenger and nascent ribosomal subunits across the nuclear envelope. (Nuclear pores allowing free passage of small and medium sized molecules, but not larger ones and macromolecular complexes³², probably arose in response to the need to regulate nucleocytoplasmic exchange.) Nucleocytoplasmic separation would have imposed new selective forces on DNA and RNA. Though it is premature to speculate on their nature I predict that they will go far to explain other differences between nucleocytoplasmic genetic systems on the one hand and prokaryote/mitochondrial/plastid ones on the other.

The origin of the unique features of cytoplasmic ribosomes and of nucleoli probably dates from the time when the pre-alga became completely compartmented to form the first true eukaryote—the proto-alga (Fig. 5). I suggest that originally the pre-algal plasmids and nucleoid had identical ribosomal DNA present in multiple tandem copies (many copies were needed because of the large cell size), and that this identity was maintained by recombination involving a ribosomal DNA episome (replicating as a rolling circle³³). But, after complete compartmentation, DNA and ribosomes could no longer cross the membranes (but proteins could be secreted directly across them by membrane-bound ribosomes³⁴), so plasmid and nuclear ribosomal DNA then evolved independently. This predicts that gene amplification and rolling circle replication of nucleolar DNA will be found universally in eukaryotes. The existence of a distinct nucleolus is connected with the need to transport both ribosomal and messenger RNA across the envelope.

Nucleocytoplasmic separation, the breakup of operon-like gene clusters and the production of large amounts of redundant DNA as suggested above, were probably enough to lead to completely novel systems of gene regulation in eukaryotes³⁶ which would subsequently make possible the evolution of highly differentiated multicellular organisms. In future research it will be important to determine, by careful comparison of primitive unicellular eukaryotes and differentiated multicellular ones, which features (for example, giant heterogeneous nuclear RNA³⁵, repetitious DNA³⁶, palindromes³⁷) are universal features of eukaryote genetic systems and which are specifically associated with complex multicellular differentiation.

Comparison with symbiosis theories

My model is superior to symbiosis theories in three main ways. First, it explains how eukaryote nuclei evolved. In doing so it provides a plausible selective advantage for the evolution

of all major features of eukaryote nuclear structure and genetic systems (except the presence of histones). According to my theory the absence of sex in an entire major eukaryote group, for example euglenoids³⁸, is the result of secondary loss and not a primitive feature. The absence of histones in dinoflagellates³⁹ might also be a secondary feature perhaps resulting from the loss of sex. If so the primary function of histones could have been efficient packing of DNA to facilitate meiotic pairing and segregation.

Second, it gives plausible reasons for the differences between nucleocytoplasmic and prokaryote/plastid/mitochondrial genetic systems. This the symbiotic theories do not do; they ignore the fact that both kinds of genetic systems must have evolved from prokaryote systems, and so resemblances are not at all surprising²¹. The real problem is why the nucleus is different, which symbiosis does not explain.

Third, it is far simpler than any symbiotic theory. These postulate two to five separate symbiotic events¹⁶. Though the symbiotic origin of mitochondria and chloroplasts is a possibility (and one compatible with the origin of nuclei by the mechanism proposed here), the resemblances between them and prokaryotes are to be expected on either theory. The symbiotic origin of flagella and the mitotic apparatus^{7,12}, is untenable for many reasons^{8,24}. The idea that the complexities of sex and meiosis evolved independently 27 times (Fig. 2–6 of ref. 7) in much the same way is incredible.

Eukaryote diversification

I regard the absence of flagella in red algae (possibly also in higher fungi if they evolved from them by plastid loss⁴⁰) as primary^{20,21,24}; they are probably the most primitive living eukaryotes. Though some amoebae without flagella could have evolved from primitive red algae by plastid loss, most eukaryotes must be derived from a red algal line which evolved 9+2 flagella from aggregates of microtubules. The selective advantage is obvious: they could colonise a completely empty niche as the first marine phytoplankton (or if, unlike today²⁵, there were then blue-green algae in the open sea, become their first predators) through their new ability to stay in suspension and migrate to the level most suitable for photosynthesis and (or) predation. By loss of phycobilins and the development of other pigments a great variety of brownish and green phytoflagellates were formed^{39,41,42}. These were the ancestors of all plants and, by plastid loss, of non-photosynthetic protists and animals (Fig. 5). The generality of the circadian clock in eukaryotes (and possible absence in bacteria) is easiest to understand if it evolved in a photosynthetic common ancestor to maximise photosynthesis by day and division by night. Complex polysaccharide–protein cell walls or surface coats, often secreted by the Golgi apparatus, evolved in many lines. Adhesion between such extracellular layers led to multicellularity, in many separate lines^{43,44}.

Comparative studies clearly indicate that centrioles are derived from basal bodies⁴⁵ and probably became secondarily associated (perhaps on several independent occasions) with the spindle poles, and are not essential for mitosis^{24,46}. Chromosome-to-pole spindle microtubules probably evolved long after the origin of mitosis, but only in some eukaryote groups²⁴. Microtubules have also independently evolved into many other organelles of motility, such as axostyles⁴⁷, axopods⁴⁸, suctorian tentacles⁴⁹ and haptonemata³⁹ as well as numerous structural elements as in the cortex of protist cells^{39,50}, the phycoplast⁵¹ or neurotubules⁵².

My view that the origin of all eukaryotic organelles and genetic systems can be traced back to two fundamental innovations, (1) cytosis involving actomyosin and (2) microtubules, has many implications (too numerous to discuss properly here) which can be tested by observations on living organisms. Unfortunately evidence from the fossil record^{42,53} will be very hard to come by because the ancestral eukaryotes had no cell walls and because the changes postulated could have occurred very rapidly indeed, possibly in a very restricted locality.

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articles

Palaeolithic remains at the Hadar in the Afar region

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Studies of Plio-Pleistocene deposits along the Awash River in the Hadar region of Ethiopia have revealed for the first time several Palaeolithic sites. In addition to a large number of artefacts which provide evidence of early to late Stone Age industries the deposits have also yielded a rich vertebrate fauna including what may be the earliest hominid remains yet discovered.

NEAR the confluence of the rivers Awash and Hadar, about 60 km ESE of Bati, the latter river has cut deeply into the gravel covered surface of the floor of the Ethiopian Rift (see Fig. 1) and has exposed 105 m of fluvial, lacustrine and tuffaceous sediments of Plio-Pleistocene age.

The flat surface of the rift floor consists of an extensive boulder gravel, 3–6 m thick, which lies unconformably on the Upper Pliocene and Lower Pleistocene sediments of the Hadar Series. This gravel extends from the foot of the Ethiopian Rift escarpment in the west, thinning slowly to beyond the Awash River in the east¹. It seems to have been deposited as a series of large confluent fans derived from the escarpment, which form an extensive behada or peripediment. It is not cemented and consists of well rounded to subrounded boulders and pebbles 30–40 cm in size, set in a matrix of sandy, calcareous silt. The pebbles are neither sorted nor oriented into any stream direction. The gravel is probably of Middle Pleistocene age, as indicated by Early Stone Age (ESA) implements found *in situ*; no fauna has yet been found.

Beneath the boulder gravel the Hadar has exposed a 105-m section of the Hadar sediments—Upper Pliocene and Lower

Pleistocene fossiliferous beds of sandstones, sands and clays, and occasional tuffs. A very rich vertebrate fauna indicates a relative age of more than 3 Myr for these beds².

Fossils of *Australopithecus*, at least 3 Myr old³, were found for the first time during the survey of the Lower Hadar sediments by members of the 1973 International Afar Expedition. These included three fragments of femur and one fragment of tibia from a small hominid and a piece of cranium (mastoid) from a more robust *Australopithecus*.

In the Hadar area the terraces of the Awash River are not very pronounced. The Awash meanders in a flood plain about 1 km wide. In the inner parts of the meanders the silty and fine-sandy, modern alluvial plain is covered by a thick riverine forest comprising various species of acacia and tamarisk. The height of the plain is not more than 2 m above low water level.

A terrace 3–5 m high runs along the inner meanders beyond the riverine forest. It is covered by a thin, bouldery gravel within a sandy matrix. The same terrace also stretches along the Denen Dora tributary further west. There, a broad terrace covered by large boulders and pebbles extends between the Denen Dora and the Sidi Hakoma tributary, rising to 4 m above the bed of the Denen Dora.

Any pre-existing higher terraces of the Awash and the Hadar have been destroyed by erosion. Significantly, many—though not all—of the Badland Hills, 25–90 m in height along the Awash, have a covering of loose, well-rounded boulders in a sandy-silty matrix lying unconformably on earlier Hadar deposits.

All the boulders which cover the river terraces and erosional surfaces in the Hadar area have been derived from the erosion of the Middle Pleistocene boulder gravel. The pebbles of this

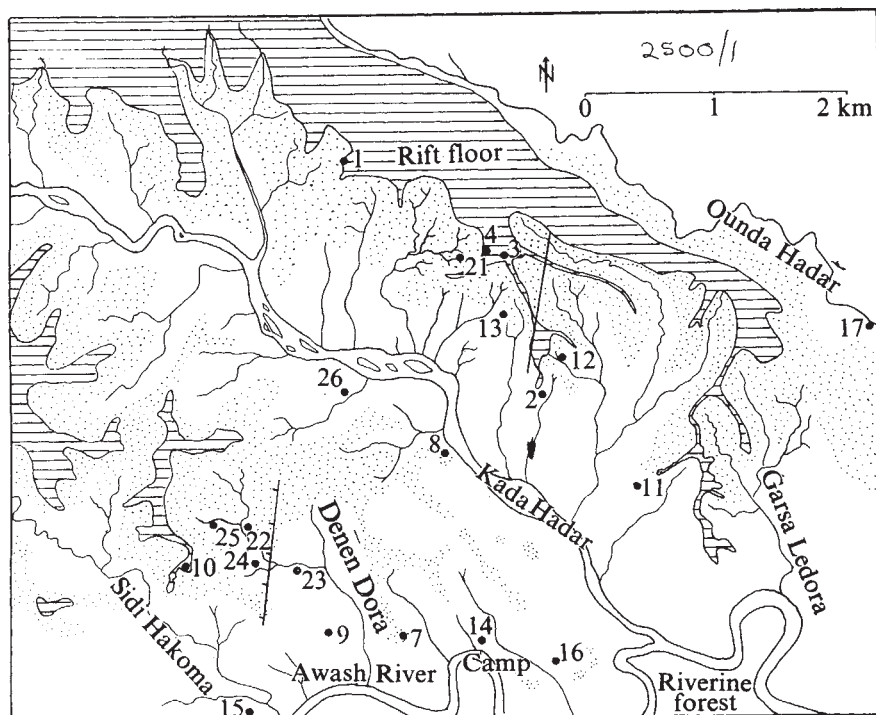


Fig. 1 Sketch map of the Hadar region. Stippled areas, Badland Hills; horizontal lines, rift floor more than 100 m above the Awash River; long straight lines, faults; ●, artefact localities.

boulder gravel consist of amygdaloidal basalt, trachyte, rhyolite and ignimbrite, derived from the Tertiary Trapps of the Ethiopian Plateau. They have a red-brown to dark-brown polish when they have been exposed for a long time. The boulders on the erosional surfaces and terraces are of the same type but they often have a deeper, sometimes black polish.

We may assume that the boulder cover of the Badland Hills represents a residue of boulder spreads which were redeposited on erosional surfaces during the downcutting of the area and which were originally derived from the boulder gravel of the Ethiopian Rift floor.

Artefact sites

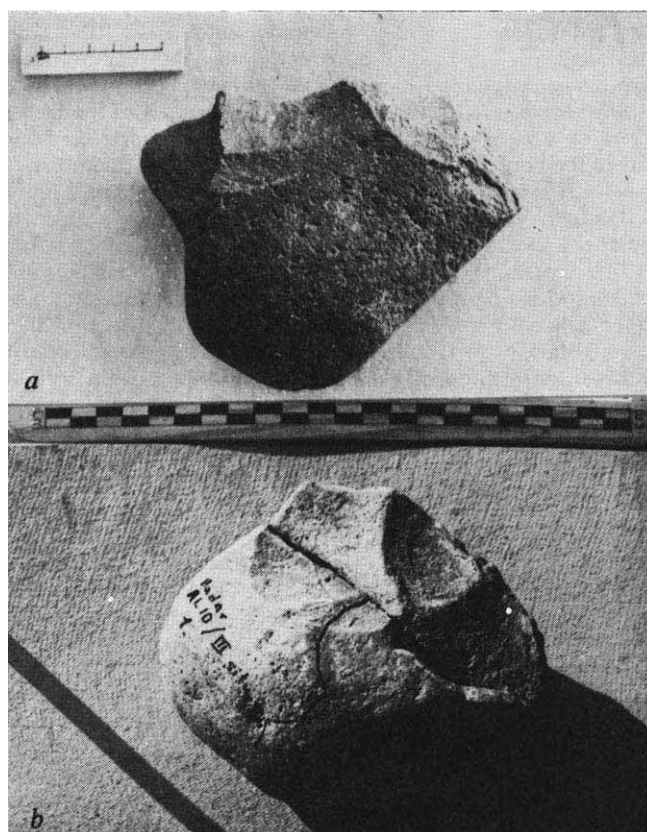
Artefacts of all ages occur everywhere in the region (Fig. 1) both on the surface and in the river gravels. At some localities up to 45 specimens were found. They occurred mostly on the surface and were rarely *in situ*, except for a few Middle Acheulian bifaces found in recent, reworked river gravels. In most cases the artefact assemblages were mixed; that is, Middle Stone Age (MSA) flakes and cores have been found together with Late Stone Age (LSA) flakes, cores and waste. Choppers and bifaces also occur occasionally among them. The bifaces, choppers, modified pebbles, and the MSA flakes and cores, are all made from the local boulders of trachyte and basalt. The LSA people however, used siliceous material, such as jasper, chalcedony and, rarely, fine grained basalt. The subrecent specimens are of obsidian and quartz.

A few sites, however, contained assemblages of an homogeneous character. Sites Hdr 9 and Hdr 22 (Fig. 1) are Acheulian sites situated on the low terrace of the Denen Dora tributary. Bifaces were found at site Hdr 9 on the surface of the wide, flat, 4-m terrace of the Denen Dora near the confluence with the Awash. At site Hdr 22 the assemblage was found on the 1.50–3.00-m recent terrace in the middle part of the stream. The bifaces from that site do not, on the whole, have a dark-brown polish but have a grey to white, calcareous patination or no patination at all. They must therefore have been eroded out from the bouldery deposit of the terrace, rather than having been brought down from a higher level; most of the other pebbles on the surface and hill slopes are polished, having been exposed for a long time. The majority have been rolled down from the surrounding hills but no artefacts were found amongst them there. It is therefore necessary to ascertain from where the tools are derived. The Acheulian bifaces are all quite fresh

and show no evidence of river transport. But a few artefacts which are quite rolled and of a rather primitive appearance represent evidence of an older industry elsewhere.

I surveyed the whole of the Denen Dora river up to the source at the escarpment of the Ethiopian Rift floor and noticed that no artefacts could be found upstream of site Hdr 22. This suggests that the tools from site Hdr 22 were not brought down from the boulder gravel covered rift floor by the Denen Dora

Fig. 2 Bifacial choppers from site Hdr 10-III: a, from surface; b, found *in situ*.



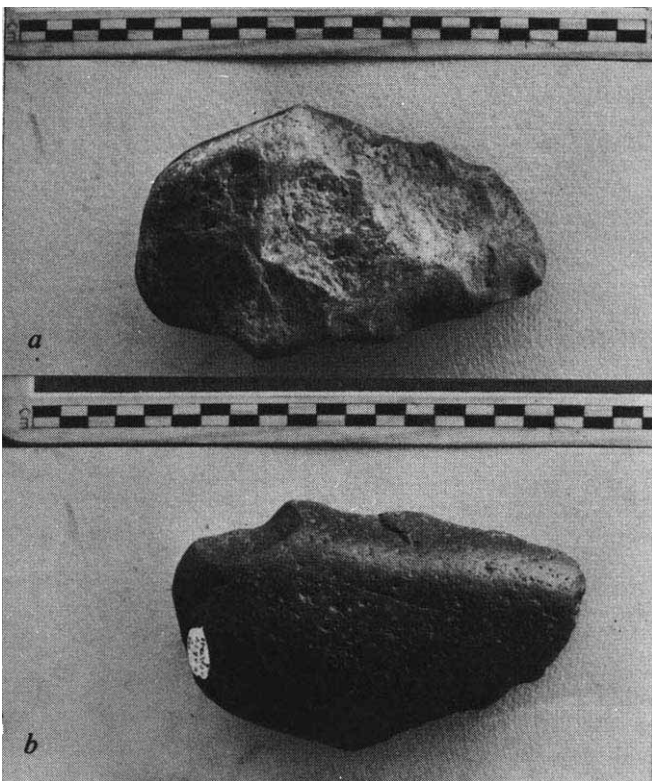


Fig. 3 Unifacial protohandaxe from site Hdr 22; *a*, ventral face; *b*, dorsal face, which is of cortex, showing the typical black polish.

River, but must derive from nearby, the original site having been destroyed.

A few very rolled, primitive bifaces and a chopper, however, were found in a small stream bed, which joins the Denen Dora from the west and which descends directly from the rift floor at site Hdr 10 (Fig. 1).

There is, therefore, evidence that at least two different types of industry were developed along the Denen Dora river. The first dates from the Middle Acheulian with rather fresh, well-made bifaces. The second, apparently older industry produced protohandaxes, choppers and crude flakes of rolled appearance.

Site Hdr 10 is situated immediately to the west of the Denen Dora on a narrow remnant of the otherwise eroded rift floor, where the boulder gravel is 3–4 m thick above the Hadar Series. At site Hdr 10-III, within an area of about 50 by 70 m, I found 32 second type artefacts on the surface and one within the boulder gravel.

These artefacts, lying among a large number of boulders and pebbles with a light-brown to dark-brown polish, seem to have come originally from within the boulder gravel. Most of them have a dark-brown polish, some of them only a light polish, and the *in situ* chopper has no polish at all but a thin crust of white lime like that on the pebbles within the boulder gravel. The artefacts comprise choppers, polyhedral and modified pebbles, crude flakes and cortex flakes and primitive handaxes or protohandaxes.

The few second type artefacts in the Denen Dora must have been brought down from the boulder gravel of site Hdr 10-III. At sites Hdr 10-I and II, I also found a few crude artefacts, some *in situ* at the edge of the boulder gravel; I dug two trial trenches into the gravel, one at each site to verify the origin. I excavated nine artefacts from the small trenches and I found eight *in situ* in the gravel along the edges. Forty-four artefacts were found on the surface.

Artefacts

Artefacts found in the Hadar area can be placed in five categories. First, at sites Hdr 10-I, II, III, (and occasionally from sites Hdr 22, Hdr 24 and Hdr 25) there were choppers, polyhedral

and modified pebbles, crude flakes and protohandaxes; second, mainly at sites Hdr 22, Hdr 23, and Hdr 9, were found bifaces and a few flakes of the Middle Acheulian type (this category includes occasional pieces from other localities); third, mainly at sites Hdr Camp 14, and Hdr 11 (with occasional pieces from other localities) there were flakes and cores of a Middle Stone Age industry, mostly without retouch, struck off from prepared cores with prepared, thinned talons; fourth, mainly at sites Hdr 1, Hdr Camp 14, Hdr 15, Hdr 18 (and a few other places), there were flakes, cores and waste of a Late Stone Age industry, made of jasper, chalcedony and various other siliceous material; fifth, mainly at sites Hdr 3, Hdr 4, and Hdr 19 (and other localities), flakes, cores and waste from subrecent to recent industries, made of obsidian and quartz.

At site Hdr 10 the choppers, modified pebbles and protohandaxes are all made of Trapp material. The choppers usually have a bifacial edge, formed by alternate flaking in two directions, though sometimes they are unifacial, formed by having a few flakes struck off in one direction only; the rest is cortex (Figs 2 and 3). Interestingly, a number of choppers seem to be transitional to bifaces or unifacial handaxes. These tools display unifacial or alternate bifacial flaking at one end of the pebble in such a way that a rounded or irregular point, or a point and part of a lateral edge is produced.

Some crudely worked pieces have been classified as modified pebbles. Polyhedrons and discoids are rare. Spheroids are absent.

The protohandaxes are a special group of artefacts showing much individual variation. Ten specimens were collected from sites Hdr 10, three from the tributaries of the Denen Dora and ten from sites Hdr 22 and Hdr 9 (Fig. 3). They show a few irregular flake scars. Much cortex remains not only at the butt end, but often over large parts of the tool. The edges are not regular; and often only one edge or part of one edge is produced with an asymmetrical, often blunt, point. In rare cases there are two edges, which are irregular, zig-zag, and with no secondary trimming. Some protohandaxes may have one face made entirely of cortex, so not all of these artefacts can be called protobifaces and the term protohandaxes is preferable. There are a few small triangular forms in which only the point has been worked bifacially.

The flakes have all been struck from unprepared, large cores (Fig. 4). The talons are plain with a large angle towards the flake surface, and the bulbs are pronounced. The dorsal face often consists partly of cortex, with only one or two former flake scars. There is no preferred shape. They may have been used, though the edges are usually irregular and often blunt.

The Acheulian artefacts from sites Hdr 22 and Hdr 9 were found mostly on the surface of the low terrace of the Denen Dora, though some were found *in situ*, redeposited in the terrace gravel.

On the whole, the bifaces are well-made, trimmed by large,

Fig. 4 Dorsal faces of flakes from site Hdr 10.



shallow flaking, with more or less biconvex sections and elongate-ovate shapes. The edges run around the whole of the circumference and are rather straight with secondary trimming in the finished specimens. Characteristically, almost all of them retain a small cortical part at the butt or, rarely, on the upper face. A few prepared cores (with basal preparation) and flakes from prepared cores also belong in this assemblage.

There are, however, a number of artefacts which are much cruder in appearance and which do not belong to the Middle Acheulian. These include crude flakes of the Hdr 10 type and a few protohandaxes. As they are all found on the surface it is possible that they originated at site Hdr 10 and were transported from there by the small, steep western tributary of the Denen Dora.

The Middle Stone Age flakes occur only on the surface in the Hadar area, not only on the lower terraces but at all levels of the eroded landscape around the Hadar River and on the floor of the Rift, too. It is, therefore, impossible to say anything about their stratigraphic context, or their age. Unlike the younger flakes, however, they are all made of basalt and trachyte and they may well be products of an Acheulian light-duty industry.

These flakes have been struck from prepared cores. The dorsal faces show primary flake scars and in many cases the talon has also been prepared. Evidence of retouch is extremely rare and any that does occur is possibly a result of chipping or battering by natural agencies during transport rather than intentional retouch. Many of the flakes are quite rolled. The shapes are mostly irregular, often with blunt edges so that a great part of them seems to be waste. But there are a few parallel-sided and pointed flakes. Cores are not abundant. There are rare tortoise-shaped cores, with a base formed by one or two flake scars; cores with a bifacial edge, with flakes removed alternately along the edge; cores with two platforms at right angles; and cores with two parallel platforms.

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Integration of viral genomes

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DNA transcripts of infectious RNA viruses were found to be integrated in the DNA of chronically-infected tissue cultures. DNA sequences homologous to RNA of measles virus were found in tissues affected with systemic lupus erythematosus. These data open up a new class of virus-cell interaction that may be a result of cooperation between infectious and oncogenic viruses.

Two types of interaction between the virus and the infected cell are characteristic of virus infections: autonomous replication of the virus in the cell and integration of the viral genome into the host cell chromosome.

Until now, integration has been found only with the temperate bacteriophages, oncogenic viruses and the related 'slow' viruses. We have shown, however, that nuclear DNA of avian, mammalian and human cells can integrate genomes of classic infectious viruses such as measles virus¹ and arbovirus², and that a similar process occurs in systemic lupus erythematosus, an autoimmune disease of man³. Here I present the results of a study of integration processes both in artificial tissue culture and in clinical samples.

To study the molecular mechanisms of the persistence of virus in chronically-infected tissue cultures three systems were used. Details of the systems, chick embryo fibroblasts infected with measles virus (CEF-MV), mouse L cells infected with Sindbis virus (ML-SV), and human Hep-2 cells infected with tick-borne encephalitis virus (Hep-2-TBEV), are given elsewhere^{4–12}. Briefly, the chronically-infected cell lines were obtained by inoculating the cultures with low doses of virus (0.01 TCD₅₀ per cell of measles virus and 0.01 plaque-forming units (PFU) per cell of the arboviruses) with subsequent reimplantation of the cultures every 4–6 d. The CEF-MV line has now undergone more than 120 passages; ML-SV, more than 80 passages; and Hep-2-TBEV more than 600 passages. All three cultures show no signs of cytopathic changes and the viruses are regularly detected in the cytoplasm by immunofluorescence tests with virus-

specific sera. Infectious titres of intracellular viruses are low (10²–10³ TCD₅₀ per ml for MV; 10²–10³ PFU ml⁻¹ for SV; and 10³–10⁴ for TBEV) and those of extracellular viruses even lower (1–10 for MV; 0–1 for SV; and 10–10² for TBEV).

Biophysical studies showed that in all three systems sub-viral structures are predominant in contrast to acutely-infected cells which produce mature viruses (Fig. 1). The specificity of viral ribonucleoproteins was confirmed by titration of infectivity.

These data were obtained in experiments where actinomycin D was added for 3 h. When the antibiotic was added for 12 h, the synthesis of virus-specific structures was strongly inhibited in chronically-infected cells, in contrast to acutely-infected cells, where the synthesis of virus-specific structures was resistant to the action of the antibiotic (Fig. 2). Similar data were obtained in experiments with measles and Sindbis viruses.

As actinomycin D inhibits the transcription of cellular DNA into RNA, DNA sequences homologous to the viral RNAs were looked for in the chronically-infected cells. ³H-labelled RNA was hybridised with excess DNA from chronically-infected and uninfected cells. DNA from chronically-infected cells was found to contain sequences homologous to RNA of the corresponding viruses whereas no such sequences could be found in non-infected cells (Fig. 3). DNA preparations from chronically-infected cells were then used to treat appropriate sensitive cells to see whether virus could be isolated from these treated cells. TBEV was isolated from SPEV cells treated with DNA from Hep-2-TBEV cells (Table 1), and SV from the brains of mice inoculated with DNA from ML-SV cells. In both cases DNase destroyed the infectivity of the DNA preparations whereas RNase had no effect.

As none of the three viruses under study possesses virion-associated or virus-induced reverse transcriptase, the transcription of virus RNA into double-stranded DNA must have been accomplished by latent oncornaviruses present in the cultures. The chickens used as a source of the CEF-MV

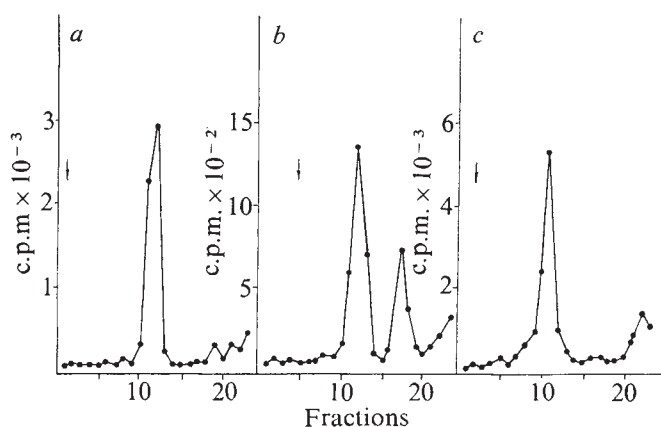


Fig. 1 Sedimentograms of virus-specific structures produced by the cultures chronically infected with measles (a), Sindbis (b) and tick-borne encephalitis (c) viruses. The cultures were treated with actinomycin D ($2 \mu\text{g ml}^{-1}$) at zero time, then ^3H -uridine ($20 \mu\text{Ci ml}^{-1}$) was added 1–3 h after treatment with the antibiotic. Thereafter the cells were disrupted in TNE buffer (Tris HCl 0.01 M pH 7.4, NaCl 0.1 M, EDTA 10^{-3}) by a Dounce homogeniser, the nuclei and mitochondria were removed by centrifugation ($15,000g$, 20 min), the cytoplasmic extracts were layered on sucrose density gradients 15–40% (w/v) prepared in TNE buffer, centrifuged in a SW 27.1 rotor of a Spinco L 3-50 centrifuge at $122,500 \text{ r.p.m.}$ for 1 h 45 min (a) of 2 h (b, c), and acid-insoluble radioactivity of the gradient fractions was determined in a Packard-Tricarb liquid scintillation counter. In parallel experiments VERO cells were infected with measles virus, chick embryo fibroblasts with Sindbis virus, and SPEV (swine) cells with tick-borne encephalitis virus. The cultures were grown without actinomycin D, labelled with ^3H -uridine ($10 \mu\text{Ci ml}^{-1}$) overnight; the viruses were collected from the culture medium; the cell debris removed ($15,000g$, 20 min); the viruses were pelleted ($120,000g$, 2 h) and purified by equilibrium centrifugation ($25,000 \text{ r.p.m.}$, 16 h) in sucrose density gradients 20–60% (w/v) prepared in TNE buffer. The gradient fractions that corresponded to the density of viruses studied ($1.22\text{--}1.24 \text{ g ml}^{-1}$) were collected and subjected to velocity centrifugation as indicated above. The arrows show sedimentation peaks of virions in the gradients parallel to a, b, and c.

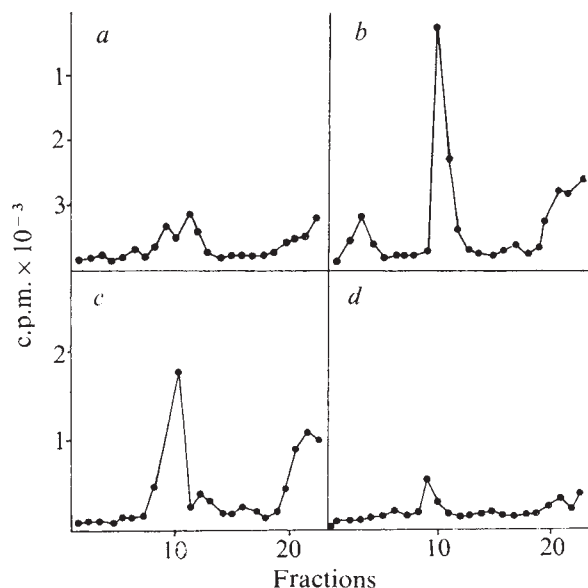


Fig. 2 Sedimentograms of virus-specific structures produced in SPEV cells acutely infected (a, b) and in Hep-2 cells chronically infected (c, d) with tick-borne encephalitis virus. SPEV cells were infected with the virus (10 PFU per cell), treated with actinomycin D ($0.5 \mu\text{g ml}^{-1}$) at zero time and incubated for 3 (a) and 12 h. (b) ^3H -uridine ($20 \mu\text{Ci ml}^{-1}$) was added 1 to 3 h after infection (a) and 10 to 12 h (b) after infection. Hep-2-TBEV cells were treated with actinomycin D ($0.5 \mu\text{g ml}^{-1}$) at zero time and ^3H -uridine was added 1 to 3 h (c) and 10 to 12 h (d) after treatment with the antibiotic. Cytoplasmic extracts were obtained and studied by velocity centrifugation in sucrose density gradients in the same way as described in the legend to Fig. 1.

cell line usually contain endogenous C-type oncornaviruses¹³, Hep-2 cells have been reported to contain a new oncornavirus¹⁴, and L cells are known to produce an endogenous C-type oncornavirus¹⁵. We carried out experiments to reveal latent oncornaviruses using a test that reveals reverse transcriptase activity associated with high molecular weight (60–70S) RNA¹⁶. Such reverse transcriptase activity was shown in all three chronically-infected tissue cultures, and also in uninfected L and Hep-2 cells but not in uninfected primary chick embryo fibroblasts.

These results suggest that during the prolonged persistence of infectious viruses in cells containing latent oncornaviruses, the two types of viruses interact, resulting in the transcription of the infectious virus RNA genomes into double-stranded DNA which is then integrated into the genomes of the chronically-infected cell. The main molecular mechanism of virus persistence in these cultures is

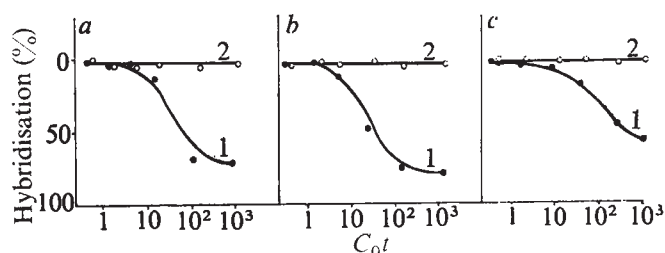


Fig. 3 Hybridisation of ^3H -labelled RNAs of measles virus (a) with DNA from chronically infected (●) and uninfected (○) chick embryo fibroblasts; tick-borne encephalitis virus (b) with DNA from chronically infected (●) and uninfected (○) Hep-2 cells; Sindbis virus (c) with DNA from chronically infected (●) and uninfected (○) L cells. The viruses were labelled with ^3H -uridine, ^3H -adenosine and ^3H -guanosine ($50 \mu\text{Ci ml}^{-1}$, $25 \mu\text{Ci ml}^{-1}$ and $25 \mu\text{Ci ml}^{-1}$, respectively) and purified as described in the legend to Fig. 1. RNA was extracted with sodium dodecyl sulphate (0.5%) and phenol, precipitated with ethanol and stored at -20°C . Specific activity of the RNA preparations was 2×10^6 to $6 \times 10^6 \text{ c.p.m. } \mu\text{g}^{-1}$ and more than 80% of RNA sedimented in the region of 50S (measles) or 42–45S (Sindbis, tick-borne encephalitis). DNA from chronically-infected cells was extracted with phenol after treatment of the nuclear fraction of the cells with 0.2% SDS and Pronase ($200 \mu\text{g ml}^{-1}$) at 37°C for 3 h. DNA was fragmented in an MSE ultrasonic disintegrator; RNA admixtures were hydrolysed by 0.5 M NaOH (37°C , 16 h) with subsequent neutralisation; DNA was precipitated by ethanol and stored at -20°C . The hybridisation mixture contained in 0.1 ml: Tris HCl 0.02 M pH 7.4, NaCl 0.6 M, EDTA 10^{-3}M , SDS 0.05%, RNA 2,000 c.p.m., DNA 1.5–2.5 mg ml^{-1} . The mixture (1.2 ml) was incubated at 68°C ; aliquots (0.1 ml) were taken at various intervals and stored at -20°C to the end of the experiment. Then the samples were diluted with 3 ml of TN buffer (Tris HCl 0.01 M pH 7.4, NaCl 0.4 M), treated with pancreatic ribonuclease ($50 \mu\text{g ml}^{-1}$) at 37°C for 1 h and acid-insoluble radioactivity was determined. RNA alone and RNA with calf thymus DNA incubated at the same temperature were taken as controls for self-annealing and nonspecific annealing. The corresponding radioactive counts (that were less than 100 c.p.m.) were subtracted and the % ribonuclease-resistant RNA-DNA hybrids were calculated and plotted against the corresponding C_0t values.

Table 1 Transfection of tick-borne encephalitis virus with DNA from Hep-2-TBEV cells

No. of experiments	Isolation of virus after treatment of SPEV cells with DNA from Hep-2-TBEV cells		
	Native DNA	DNA treated with RNase ($300 \mu\text{g ml}^{-1}$)	DNA treated with DNase ($200 \mu\text{g ml}^{-1}$)
1	$10^{3.5}$	$10^{3.1}$	0
2	$10^{3.3}$	$10^{3.4}$	0
3	$10^{3.2}$	$10^{3.0}$	0

SPEV cells were treated with DEAE-Dextran (0.1%) for 10 min, then with DNA ($200 \mu\text{g}$) from Hep-2-TBEV cells, thereafter culture medium was added and the cultures were observed until cytopathic changes appeared. The figures indicate the titre of the virus (PFU ml^{-1}) in the culture medium. The specificity of the virus strains isolated was determined by plaque titration and neutralisation with immune serum and by intracerebral inoculation of mice.

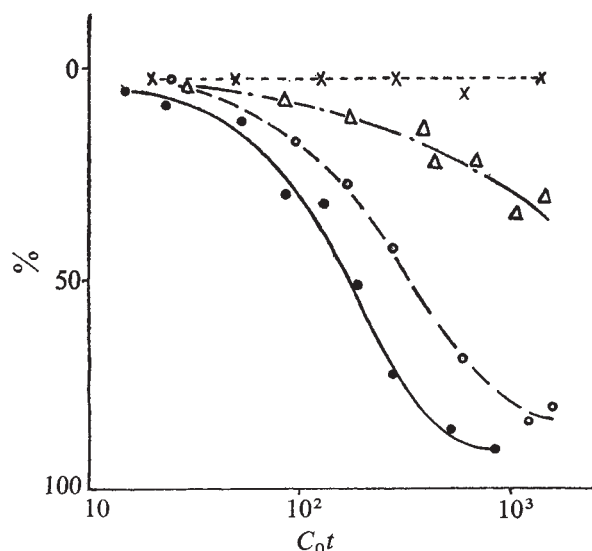


Fig. 4 Kinetics of hybridisation of ^3H -labelled measles virus RNA with DNA from leukocytes (Δ), lymphatic node ($\bullet$), and kidney ($\circ$) of a patient and of a fatal case of systemic lupus erythematosus and with DNA from normal embryonic human spleen ($\times$). The methods of preparation of ^3H -labelled measles virus RNA and of DNA from tissues as well as techniques of hybridisation were the same as described in the legend to Fig. 3. Hybridisation was carried out in the volume of 1.2 ml; aliquots (0.1 ml) were taken at various intervals and the percentage RNA hybridised was plotted against the corresponding C_0t values.

therefore the production of subviral structures, whose production is inhibited by the prolonged addition of actinomycin, by the cell genome, rather than the autonomous replication of virus particles, which are produced shortly after addition of the antibiotic.

As these models may be considered artificially induced, similar natural pathological processes were looked for. Systemic lupus erythematosus (SLE) provides a suitable study system. The disease is characterised by high titres of measles antibodies which correlate with the pathological course of the disease¹⁷, by the presence of measles antigens in leukocytes detected by immunofluorescence¹⁸, and by tubular structures in the cytoplasm of SLE-affected tissues resembling nucleocapsids of paramyxoviruses¹⁹. ^3H -labelled measles virus RNA was hybridised to DNA from tissues and leukocytes from SLE patients and fatal cases. Sequences homologous with measles virus were detected in the DNA from these tissues (Fig. 4). No such sequences were detected in DNA from normal human tissues and in

DNA from leukocytes of measles patients taken at an early stage in the disease and in convalescence.

Tissues affected with lupus erythematosus and leukocytes from patients with the generalised form of the disease were found for reverse transcriptase activity associated with high molecular weight RNA. This test is positive with cytoplasmic extracts from lupus erythematosus tissues. In parallel studies we revealed tubular structures in lupus-affected tissues (electron microscopy) and measles virus antigens in leukocytes (immunofluorescence). These results suggest the following molecular mechanism for the initial events in the development of SLE.

In comparatively rare cases of infection with measles or measles-like virus, the virus interacts with a latent oncornavirus, resulting in transcription of the RNA of the infectious virus into double-stranded DNA and integration of the latter into the cell genome. The integrated viral genome may be expressed in the synthesis of virus-specific envelope proteins that, in the case of paramyxoviruses (including measles virus) are incorporated into the cell membrane and modify its antigenic properties²⁰. Such cells become targets for attack by immunocompetent T cells and this triggers the development of the autoimmune disease.

Among human diseases similar molecular mechanisms may underlie syndromes closely related to lupus erythematosus (dermatomyositis, scleroderma, periarteritis nodosa) and also some chronic degenerative diseases of the central nervous system (subacute sclerosing panencephalitis, disseminated sclerosis).

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letters to nature

Definition of 'charge on an atom' and nature of the inductive effect

A PRECISE measure of the charge on an atom in a molecule is provided by calculating the number of electrons within a sphere of covalent radius using molecular wavefunctions. The results for alkyl substitution suggest that the simple qualitative ideas of the inductive effect may be less simple than is commonly supposed and that methyl groups are not intrinsic electron donors but rather attracters of charge.

Although the only precise 'charge on an atom' is its nuclear charge, chemists frequently use the expression for what is

more correctly the charge 'close to' an atom. Quantum mechanical calculations provide values of such quantities by using the so-called Mulliken Population Analysis¹. This has the virtue of being easily programmed for a computer, but has the defect of assigning all the charge in a molecule to particular nuclei. Nonetheless it is the origin of most of the quoted 'charges on atoms' which appear in the literature, and its well known defects such as equal division of charge between bonded centres and occasional negative populations are usually ignored.

In principle, from a molecular wavefunction, ψ , it is possible to calculate the electronic charge in any restricted region of

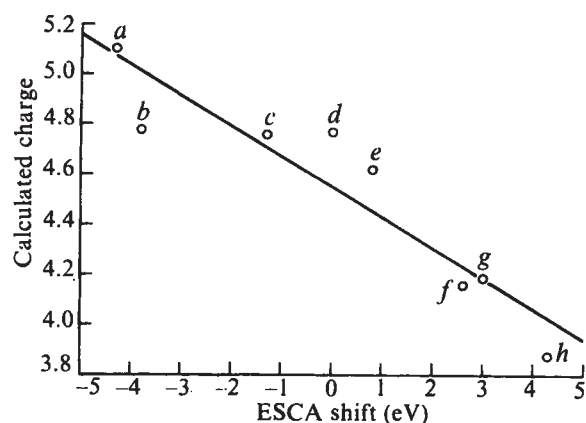


Fig. 1 Relationship between calculated charge within one covalent radius of nitrogen atoms and X-ray photoelectron shifts ($1s_N$) for: a, NH_3 ; b, CH_3CN ; c, $^*\text{NNO}$; d, N_2 ; e, NO ; f, NN^*O ; g, NO_2 ; h, NF_3 .

space by integrating the value of $\psi\psi^*$ over the defined region. This precise definition of charge in a specified region has rarely been used^{2,3} because the computation of the integrals over restricted regions of space is very difficult.

As part of our interest in the charge 'on' the nitrogen atom in the ammonium group of biologically active amines, we have overcome these integration problems by taking the interesting atom (nitrogen) as the centre of our polar co-ordinate system. The charge, q , within a sphere of radius r centred on the nitrogen atom is then given by:

$$q = \int_0^{2\pi} \int_0^{\pi} \int_0^r \psi\psi^* r^2 \sin\theta dr d\theta d\phi$$

In these calculations, the approximation has been made that the charge around an atom arises only from the integral of the following terms in the square of the LCAO expression:

$$\sum_i \sum_j (c_i^2 \chi_i^2 + 2c_i c_j \chi_i \chi_j + c_j^2 \chi_j^2)$$

where the i s are atomic orbitals of the central atom, and the j s are centred on atoms bonded to this central atom. This is an excellent approximation: terms from non-bonded atoms are very small.

Table 1 Calculated charges in substituted ammonium ions

Molecule	Charge on nitrogen atom
NH_4^+	5.054
CH_3NH_3^+	4.774
$\text{C}_2\text{H}_5\text{NH}_3^+$	4.786
$(\text{CH}_3)_2\text{NH}_3^+$	4.492
$\text{CH}_3\text{NH}_2 \cdot \text{C}_2\text{H}_5$	4.501
$\text{CH}_3\text{NH}_2 \cdot i\text{-C}_3\text{H}_7$	4.508
$\text{CH}_3\text{NH}_2 \cdot n\text{-C}_3\text{H}_7$	4.503

For each molecule we have considered, good *ab initio* molecular wavefunctions have been calculated using the Gaussian '70 molecular orbital program⁴ which takes account of all the electrons in the molecule, and overlap integrals have been calculated using the method of Barnett and Coulson⁵. The charge 'on' the nitrogen atom is defined as the electronic charge—the number of electrons—within one covalent radius (0.7 Å).

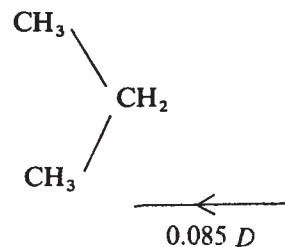
Charges were first calculated for small nitrogen-containing molecules for which X-ray photoelectron spectra (ESCA)

shifts are available⁶. The energy shifts for $1s$ -electron ionisation are thought⁷ to depend in a reasonably direct way on the charge 'on' the atom, and thus provide a means of testing the physical significance of our calculated charges. Figure 1 shows that the correlation between charge and shift is good. The Mulliken charges, in spite of the simple basis on which they are computed, give a correlation with shift which is only slightly less good.

As model compounds of particular interest to us we considered substituted ammonium ions. The calculated charges are shown in Table 1. The fact that the charge on the nitrogen atom decreases with increasing substitution of hydrogen atoms by alkyl groups is particularly significant and is in direct opposition to the traditional view that alkyl groups have a $+I$ effect—that they donate electrons, relative to hydrogen.

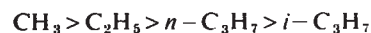
Much of the evidence from which the $+I$ effect of alkyl groups is inferred pertains to reactions and equilibria in solution: the present calculations refer to molecules in the gas phase, and so comparisons with solution data must be made with caution. But some gas-phase data are available; the most directly relevant concern dipole moments.

Laurie *et al.*⁸ have performed a series of microwave measurements of dipole moments. They have shown that the dipole moment of propane is 0.085 D with the electron drift in the direction shown:



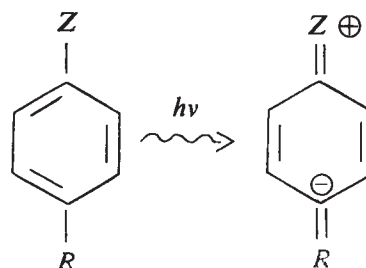
This very strongly suggests that methyl groups are in fact 'electron-withdrawing' relative to hydrogen atoms.

The dipole moments of a series of chloroalkanes are shown in Table 2. Assuming that the dipole acts in the direction $R \rightarrow \text{Cl}$, the electron-withdrawing power of the different alkyl groups, R , may be inferred to be:



This is also the order inferred from the calculated charges in ammonium ions.

Spectroscopic measurements suggest that in certain circumstances alkyl groups may act as electron acceptors rather than as electron donors⁹. The principle electronic transition in phenol, anisole, aniline and dimethylaniline in the gas phase may be presented crudely as:



Solvent effects confirm that the electron migration in these transitions is indeed away from the functional group Z and towards the *para* substituent R . Now when R is an alkyl group, the energy of the transition is lower than when R is a hydrogen atom: thus the alkyl group seems to act as an electron acceptor.

Table 2 Dipole moments of chloroalkanes¹⁰

Molecule	Dipole moment (D)
CH ₃ ·Cl	1.83
C ₂ H ₅ Cl	2.00
<i>i</i> -C ₃ H ₇ Cl	2.15
<i>n</i> -C ₃ H ₇ Cl	2.04

Ingold¹⁰ and Braumann^{11,12} have suggested a reason why alkyl groups should seem to donate electrons in many experimental situations. The gas-phase basicities of ammonium derivatives increase in the order:



This is often quoted as evidence for the +I effect of methyl groups. But Braumann *et al.*^{11,12} have measured the gas-phase acidities of the same compounds, and have found that these increase in the same order. The +I effect cannot explain both these sets of observations. Alkyl groups can, however, be polarised much more than hydrogen atoms, they are thus more able to stabilise both anions and cations, and therefore enhance both acidity and basicity.

Work by Hudson *et al.*¹³ has placed this polarisability effect on a somewhat more quantitative basis by use of a perturbation model of alkyl substitution. The influence of the alkyl group is considered to be split into two parts, one a repulsion which is greater in the neutral molecule than in the ion, and one an attraction which acts only in the ion. This treatment predicts that alkyl groups in alcohols or amines may act as either apparent donors or acceptors, depending on the conditions.

Perhaps the area in which the idea of alkyl groups as electron donors has been most widely used is in the explanation of the activating, *ortho*-*para* directing effect of one or more alkyl groups introduced into benzene. Preliminary calculations suggest that, although the *p*-toluene tetrahedral intermediate involved in electrophilic substitution is indeed lower in energy than the *meta*-substituted intermediate, the charge distribution is unlike that predicted by simple resonance bonding theory. But the situation is far from simple and it is possible that solvation effects are very important.

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A low velocity zone underlying a fast-spreading rise crest

WE present the results of an unreversed seismic refraction profile on the East Pacific Rise near the Siqueiros Fracture Zone recorded using a digital ocean bottom seismograph (OBS)¹. An analysis of *P* wave arrival times and amplitudes indicates a velocity gradient in the top 2 km, with the velocity reaching 6.7 km s⁻¹. This is underlain by a low velocity channel some

1.4 km thick in which the velocity decreases to around 4.8 km s⁻¹. Below this low velocity region there is a velocity gradient from 6.2 to 6.8 km s⁻¹ and mantle velocities of 7.7 km s⁻¹ are reached at a depth of 6 km below the sea bed.

Many surveys have been made of the structure in the neighbourhood of slow spreading ridges, particularly the Mid-Atlantic Ridge, but very little work has been done on fast spreading ridges. Two surveys^{2,3} on the East Pacific Rise near the area of this work show mantle velocities below 8 km s⁻¹.

During the course of a gravity and seismic refraction survey in the neighbourhood of the Siqueiros Fracture Zone (Fig. 1)

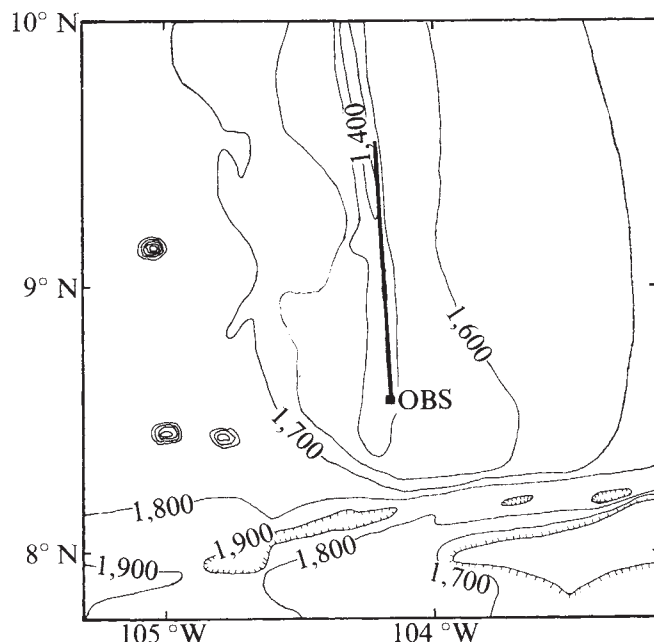


Fig. 1 Position of OBS refraction profile along the crest of the East Pacific Rise. Contours are in uncorrected fathoms.

made with the Hawaii Institute of Geophysics, a refraction profile was carried out along the crest of the East Pacific Rise, all shots being recorded on a single OBS. The local spreading half rate is high, about 60 mm yr⁻¹, and the portion of the rise crest studied consists of a horsted block about 10 km across.

A record section of this ridge crest profile is shown in Fig. 2. A reduction velocity of 8 km s⁻¹ has been applied to the section and, to facilitate amplitude studies, the traces have been normalised to a shot size of 109 kg (ref. 4). Since all shots were recorded with the same instrument, there is no problem with variability in instrumental response. The records have been passed through an aliasing filter in the OBS before digitising at 128 samples s⁻¹ and subsequently bandpassed with a zero phase 1–9 Hz filter. Timing corrections have been made for the depth of the shot and bottom topography near the shot points.

The travel times (*T*) for both first arrivals and prominent second arrivals were measured for each distance (*X*). The graphical construction technique of Bessonova *et al.*⁵ was then used to determine the delay time $\tau (= T - pX)$ for the values of the ray parameter $p (= dT/dX)$ present in the records. A smoothed spline was also fitted to the (*T*, *X*) data and estimates of the slope *p* made from this, τ values were then found by direct construction. The values of $\tau(p)$ obtained from the two methods were in good agreement. The ray parameter, or inverse phase velocity, *p* corresponds to the local slope of a travel time curve and the delay time $\tau(p)$ to the time intercept at zero distance made by a tangent to the travel time curve with this slope.

The advantages of working with the $\tau(p)$ relation are that the delay time τ is a monotonically decreasing function of the ray parameter, and that for a model containing a low velocity zone

it exhibits a discontinuity for p equal to the reciprocal of the velocity at the lid of the zone. In the present case the τ/p curve we have determined has two distinct branches separated by a jump of 0.4 s between the values for phase velocities of 6.70 km s^{-1} and 7.00 km s^{-1} , which suggests the probable existence of a low velocity zone with a lid velocity of 6.7 km s^{-1} . Analysis of sonobuoy records at short range taken on the same profile give independent evidence for a velocity of 6.7 km s^{-1} .

From our delay time results we estimated upper and lower bounds on $\tau(p)$ and then inverted these to give extremal velocity profiles using the method of Bessonova *et al.*⁵. With the adoption of an assumed lower bound on the velocity in any low velocity zone, we are able to prescribe upper and lower bounds on the depth at which a velocity can occur, outside of a low velocity zone. Travel times for models which satisfied these bounds were tested against the travel time observations, and successful models were used in the amplitude calculations. Synthetic seismogram sections were calculated for these velocity profiles using a modification of the reflectivity algorithm^{6,7} which enables the attenuation structure to be included. Although a large number of models could be found to fit the travel times, the relative amplitude of arrivals along the records proved an effective discriminant.

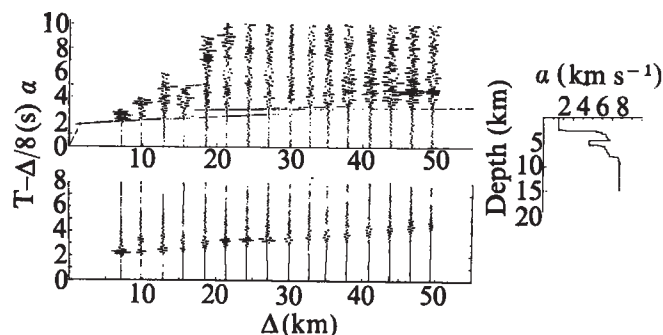


Fig. 2 Seismic profile and interpretation. Top figure shows the data with travel time curve corresponding to model at right. Bottom figure shows synthetics corresponding to model.

The model presented in Fig. 2, together with the corresponding synthetic seismogram section, was the best fit to both the travel time and amplitude criteria. Several attempts to model the observed records without a low velocity zone proved singularly unsuccessful with regard to the amplitude criteria. Models without low velocity zones also yielded crustal thicknesses significantly greater than any measured in ocean basins. We have assumed that all attenuation arises in shear⁸ and adopted a shear wave Q of 175 in each of the layers, except in the low velocity channel where it was dropped to 100, since this improved the fit to observations. Unfortunately, we have no independent evidence to determine attenuation values. It should be noted that the amplitude of the first arrival between 30 and 50 km decreases too slowly for the transition to a 7.7 km s^{-1} layer to be a discontinuity, but the behaviour can be modelled quite well with a gradient. Even our best model does not fit the amplitude data exactly. Factors contributing to this difficulty in fitting the data include the inexactness of the normalisation and the likelihood of lateral variations along the profile.

The low velocity, lower Q zone beneath the ridge crest is hypothesised to be a zone of partial melt or a magma chamber as predicted by Cann^{9,10}. This low velocity material is underlain by high velocity crust, and there is evidence of a low velocity 7.7 km s^{-1} mantle. Our observation of a mantle arrival requires any intrusion zone to be narrower than about half a wave length (0.7 km) wide at the mantle interface. This low velocity mantle is in agreement with most previous work at spreading centres¹¹⁻¹³.

For profiles conducted on the flanks of the rise crest during the same survey, mantle velocities in excess of 8 km s^{-1} were only detected when the crustal age exceeded 5 Myr.

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Mercury contamination in a 54-m core from lake Huleh

THERE has been some discussion¹ concerning the suggestion of Aston *et al.*² that recent technological growth has resulted in a progressive increase in mercury in the sediments of Lake Windermere, UK. Here I report some data from another lake to substantiate their suggestion.

A 54-m core³ was taken in the deepest portion of the now drained Lake Huleh located in the upper part of the Jordan Valley. The sediments are indicative of a swamp which underwent small periodic changes in level and was converted to a lake, presumably by the damming of the Jordan River by the late Pleistocene Yorda Basalt some 30,000 yr b.p. Before the great rise in water level reflected in the upper lacustrine series, there are two small lacustrine episodes when the water must have been transiently too deep to deposit peat. Figure 1 shows the gross stratigraphy of the core.

The concentration of Hg in the Huleh sediments is highest in the surface mud of the modern lake (Fig. 1 and Table 1), whereas that in the second and first lacustrine periods is lower. Forty duplicate samples of each level ($n = 746$) were analysed for Hg by optical emission spectroscopy^{4,5}. These results were confirmed by another laboratory using flameless atomic absorption spectroscopy. Mercury concentrations in the Huleh mud ranged from 2.36 to 0.11 p.p.m. with a mean of 0.36. The percentage relative s.d. for high values was 0.62 and that for low ones was 3.1 (ref. 4).

In the first 137 cm of the core, the Hg distribution follows that of Fe (ref. 6) (Fig. 1 and Table 2). The concentration of Hg begins to decline about 122 cm. From 142 cm to the beginning of the peat (4,572 cm) the distribution of Hg follows that of organic matter, here measured as percentage loss of weight on ignition³. There is a significant relationship between Hg and organic matter in the peat zones but the Hg concentration is more variable in this type of material. Although plant fragments prevalent in the more recent sediments may contribute to the Hg content⁷, it would be presumed that this would also be the case in the peat zones, yet the latter contain sections where there is less Hg than in the standard lake mud.

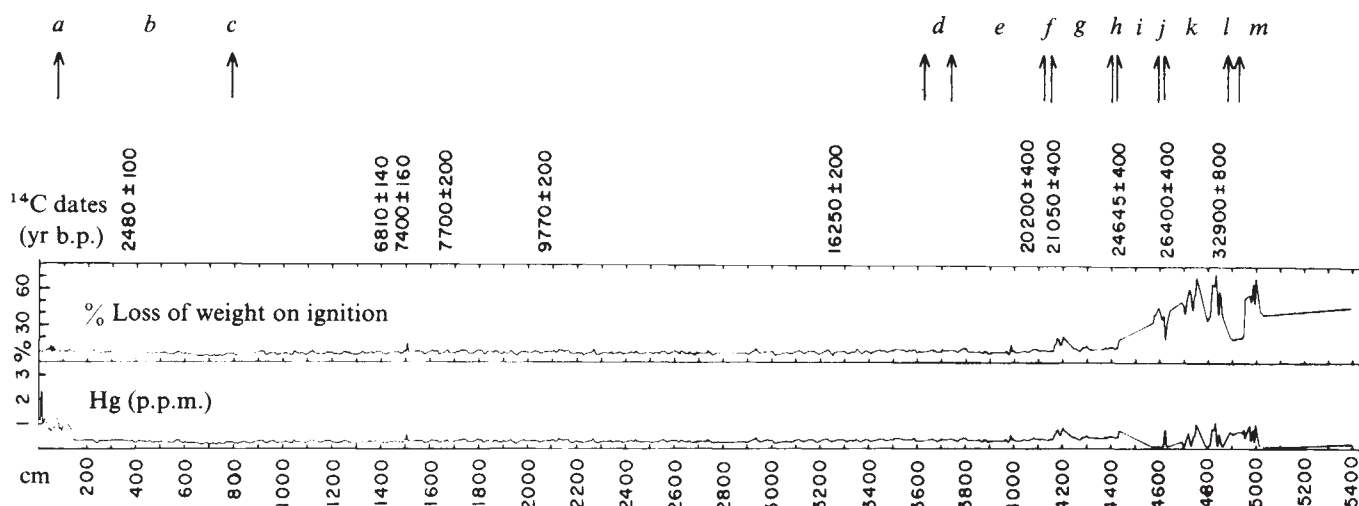


Fig. 1 Distribution of Hg and percentage loss of weight on ignition³ in a 54-m core taken from Lake Huleh. Radiocarbon dates (organic C)³ and the stratigraphy³ of the core are included. *a*, Shallow water sediments with shell and some plant remains; *b*, grey stiff lake sediment with occasional shell; *c*, grey stiff lake sediment with shell largely absent; *d*, grey stiff lake sediment with decomposed clam shells; *e*, darker grey and more organic-looking material; *f*, beginning of main lacustrine episode, material less organic; *g*, peat with shell layers; *h*, second lacustrine period, dark grey sediment; *i*, peat; *j*, less peaty; *k*, peat with shell layers; *l*, first lacustrine period, very wet grey mud; *m*, peat with shell layers.

This would suggest that the plant fragments do not contribute significantly to the Hg content of the sediments.

It would seem on the basis of the linear correlation coefficients that Hg is initially associated with Fe and detrital clay particles. This has been noted in Lago Maggiore, Italy⁸. In Lake Huleh, for the whole core⁹, quartz is associated significantly with halloysite ($r = 0.922$) and with generalised clay peak at 4.48 Å ($r = 0.884$) so that a correction for the diluting effect of quartz was not made¹⁰. The relationship between the mineralogical components of the core and Hg is significant only in the upper portion of the mud (Table 2).

Table 1 also shows the mean concentration of a few other elements that may reflect the effect of technological development. Both Ag and Cd are, in the absolute sense, more concentrated in the upper, more recently deposited mud than in the grey lake sediment beneath, although the mean rise in Ag is hardly significant. With Pb, however, the concentration at the surface is low because there is little automobile traffic in the vicinity of Lake Huleh. Presumably the oscillations in Pb reflect the natural erosion processes in the basin.

It has been suggested by some¹ and rejected by others^{2,10} that upward postdepositional migration of Hg by organisms, or through interstitial waters by ionic or molecular diffusion, or compaction of the sediment, could produce a rise in Hg concentration in the upper portions of the more recently deposited sediment. As the surface muds of the earlier lacustrine periods do not show the increase in concentration so noticeable in the recent modern surface, such upward mobility does not seem to have been significant in the Huleh core.

On the basis of radiocarbon dates³, the rise in Hg in the Lake Huleh sediments begins about 1200 AD. The first recorded mention of Hg was by Aristotle in the fourth century BC when it was used in religious ceremonies¹¹. Until the sixteenth century¹², consumption was small and chiefly for medicinal and cosmetic purposes. Before 1850 (ref. 12), two great mines dominated European production. The Almaden mine in Spain began production in 400 BC and the Idria mine in Yugoslavia was first used about 1470. Besides such activity, heavy industry, the burning of fossil fuels and sewage disposal have all contributed to the global Hg distribution which eventually finds

Table 1 Mean concentration (p.p.m.) of some elements in various portions of the Lake Huleh core

Depth (cm)	Hg	Cd	Pb	Ag	Number of samples	Stratigraphy
0-77	1.07	1.66	94.9	0.069	15	Modern Lake mud surface
82-792	0.36	0.58	97.2	0.055	91	Grey Lake mud
4,245-4,425	0.48	0.78	113.5	0.054	25	Second lacustrine period
4,890-4,941	0.65	2.49	111.5	0.11	11	First lacustrine period

Table 2 Pertinent linear correlation coefficients between Hg and other substances^{7,9} in the Lake Huleh core

Depth (cm)	Montmorillonite	Halloysite	4.48 Å peak	Quartz	Calcite	Fe	% Loss of weight on ignition
0-77	0.640*	0.918	0.922	0.910	-0.852	—	—
0-137	—	—	—	—	—	0.939	-0.221
137-792	—	—	—	—	—	—	0.987
Peat	—	—	—	—	—	-0.129†	0.956
0-5,400	—	—	—	—	—	—	0.194
Without peat	—	—	—	—	—	—	0.562
Without peat and 0-137 cm	—	—	—	—	—	—	0.982

*Not significant; †, 2% level.

Unless otherwise noted all are significant beyond the 0.1% level.

its way to the atmosphere. Such information and the data obtained from the analyses of the Lake Huleh mud certainly suggest that the increase in Hg concentration in recently deposited lake muds reflects man's global activities.

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A resonant point absorber of ocean-wave power

VARIOUS large scale systems have been proposed for absorbing and utilising the energy carried by ocean waves¹⁻⁴. In principle, such a system consists of a damped oscillator which interacts with the wave. The net result is that energy is transferred from the wave to a load. Here we call a system a 'point absorber' when its horizontal extent is much smaller than one wavelength, and a 'linear absorber' when the system is made as a straight construction, at least a few wavelengths long¹.

A progressive wave on deep water transports an energy of

$$K = (\rho g^2 / 2\omega) \langle \eta^2 \rangle$$

per unit time and per unit length of the frontage of the wave. Here, ρ is the density of water, g is the acceleration of gravity, η is the surface elevation and ω is the angular frequency of the wave. $\langle \rangle$ Represent average over a time which is typically 20 min.

For the region west of the Hebrides Salter¹ reports that K has an annual average of 77 kW m⁻¹. Although the power incident on the coast may be somewhat smaller, ocean waves represent a very interesting energy resource for the coastal countries if the energy can be converted at a reasonable cost.

We report here on a proposed point absorber which is optimised for efficient energy conversion³. The resonant frequency of such an absorber is at all times tuned to the characteristic frequency of the wave. This may be realised by incorporating into the oscillating system a flywheel with adjustable moment of inertia. The importance of this tuning facility is that at resonance, the movement of the oscillator is in phase with the dynamic pressure of the incoming wave, resulting in a substantial transfer of energy from the wave to the oscillator. At resonance the motion of the oscillator is magnified with respect to the motion of the incoming wave. So the oscillator is able to produce the oscillating fluid displacement which is required for an effective interaction between the wave and the point absorber. Thereby, in a very efficient way, a secondary, ring-shaped, outgoing wave is generated, which interferes with the incoming wave in such a way that the resulting transmitted wave carries with it less energy than the incoming wave does.

Another condition which must be satisfied to obtain optimum performance concerns the energy delivery from the resonant oscillator. If the waves are sinusoidal, the condition for optimum

power output is that equal amounts of energy are delivered to the load and to the generated outgoing waves. This means that the mechanical load resistance should at all times be adjusted equal to the radiation resistance. But for wind-generated waves, which are not purely sinusoidal, the load resistance should be somewhat larger than the radiation resistance³ to obtain maximum power output.

Resonant point absorbers having horizontal oscillating movement can be constructed, as can absorbers with vertical movement. As an example, consider the cylindrical, vertically oscillating tank of diameter $2a$ shown in Fig. 1. When the

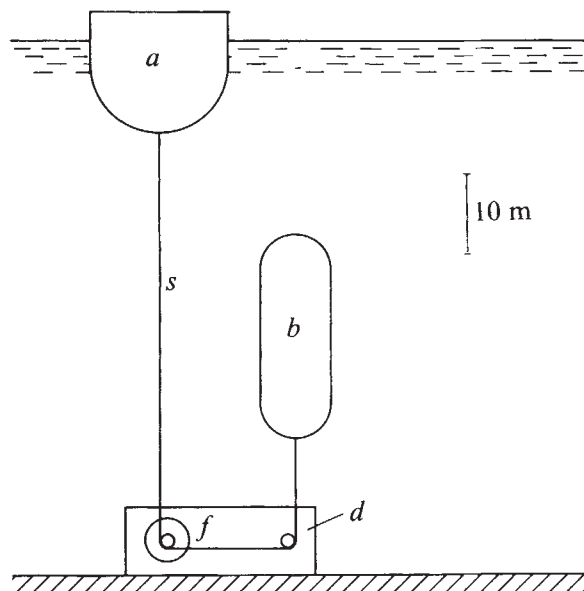


Fig. 1 An example of a single unit of a point absorber. The tank a is kept in a semi-submerged equilibrium position by means of the wire s which is stretched by the buoyancy of the auxiliary tank b . The wire s also drives a flywheel f so that it rotates in one direction when the tank a moves downwards and in the opposite direction when the tank moves upwards. The resonance frequency of the point absorber is tuned to the characteristic frequency of the wave by adjusting the inertia moment of the flywheel f which is placed in a housing d on the bottom of the sea.

resonant frequency is tuned to the frequency of the wave and the damping is optimised, then the maximum available useful power in small amplitude sinusoidal waves is³

$$P_{opt} = [(\rho g \pi a^2) / 4 R_r] \langle \eta^2 \rangle$$

Here R_r is the radiation resistance, and $\langle \eta^2 \rangle = \eta_0^2 / 2$ where η_0 is the amplitude of the wave.

We define an absorption length d_a by

$$P_{opt} = K d_a$$

Here d_a expresses the width of a wave front across which passes an average amount of power equal to that converted by the point absorber. For the shallow tank in Fig. 1 we find that in sinusoidal waves of period of the order of 10 s the absorption length exceeds 50 m even though the diameter of the tank is only 16 m. But because of the power limit of the mechanical absorber and the corresponding electric generator, and because of the fact that wind-generated waves are not purely sinusoidal, the effective absorption length is smaller. With a reasonable power limit and a wave power spectrum with relative half-value width of 0.2, we obtain³ an effective absorption length of

approximately 20 m as an annual average for waves typical of the North Atlantic.

The functioning of a point absorber is analogous to the operation of the antenna of a radio receiver. Such an antenna absorbs much more power from the wave, to which frequency it is tuned, than is incident on to its physical cross section. Correspondingly, the tuned oscillating tank has an absorption length larger than its diameter.

In contrast, a linear absorber has an absorption length which is, obviously, smaller than its physical extent. Reports on the linear absorber proposed by Salter indicate⁵ an absorption length which exceeds 50% of the length of the construction.

Since the wave energy is available in a relatively narrow spectral frequency band, the resonant point absorber is a very effective energy converter. Its physical dimensions are not prohibitively large for mooring the device. The mooring provides a steady reference against which the oscillator can act with very good efficiency.

A practical construction of a resonant point absorber will probably be different from that shown in Fig. 1. It is possible to design a system where the flywheel and the other machinery are placed inside the heaving tank. Except for a mooring wire and anchor, no installation is then necessary on the bottom of the sea. The resonant point absorber is preferably placed in an exposed location just off the coast. This condition requires that the depth is, perhaps, 50 m at the installation site.

The absorbed energy may be converted to electrical energy by a generator connected to the flywheel, and which then acts as the adjustable load resistance. Since the construction is moored, the electrical energy produced could be brought ashore by cables.

As indicated previously it is necessary that any wave energy absorber generates secondary, outgoing waves. It should be noted that practically all the volume of the heaving tank is used to displace fluid and thus to generate outgoing waves. In contrast, other proposed wave absorbing systems have a considerable amount of dead volume which does not participate in generating secondary waves.

The technological problems, including mooring, force transmission, and energy conversion seem to be manageable. A technological and economic study is, however, necessary before designing a real resonant point absorber.

If the aim is to optimise the ratio between the absorbed, converted energy and the energy in the waves incident on a coastline, the linear absorber proposed by Salter¹ is probably a better solution than an array of point absorbers. But if the aim is to convert a given amount of energy as cheaply as possible, we believe that a line of resonant point absorbers may be superior to a linear absorber. The previously mentioned, movement magnification in the resonant oscillator is probably an additional advantage when the technological details of the power converting system are being considered.

A large scale power station must consist of many, say 100, resonant point absorbers. If they are placed on a line with a distance of 100 m between neighbouring units, they will cover a coast line of 10 km. The individual units (each of 16 m diameter) will then operate practically independently of each other and absorb at least 20% of the wave power which is incident on the 10 km coastline.

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Climatic reversal in northern North Atlantic

OVER the European arctic and subarctic seas the atmospheric circulation has recently tended towards northerly airflow, with an accompanying climatic deterioration. Since the 1950s direct northerly outbreaks have swept the Norwegian–Greenland Sea with increasing frequency, adding almost every year, particularly in the 1960s, to the severity of the climate in areas as far south as the British Isles. This change has been associated with the establishment over Greenland in the early 1950s of a persistent ridge of pressure anomaly and with its subsequent maintenance and intensification (on average) throughout the late 1950s and 1960s. Compared with the ‘normal’ climatic period of 1900–39 this cell represented an increase of over 3 mbar in the mean annual pressure at sea level in Greenland over the period 1956–65, but was very much more pronounced during the cold season (November–March). During the winter quarter (December–February), the increase in the mean pressure at sea level in Greenland amounted to over 7 mbar between 1900–39 and 1956–65 (Fig. 1a) and the pressure rise continued during the late 1960s. (Throughout, listed dates refer to the second year of the winter in question; thus, “winter 1956” refers to the period December 1955–February 1956.) A further increase of over 5 mbar occurred in Greenland between the winters of 1956–65 and 1966–70 (Fig. 1b). Coupled with a slight decrease of pressure over the eastern Norwegian and Barents Seas this change has resulted in “. . . a remarkable difference of pressure between Greenland and the eastern Norwegian Sea; this has increased since 1950 in every month of the year . . .”¹.

The resulting increase in the occurrence of northerlies over the Norwegian–Greenland Sea, and the physical repercussions, have been reviewed by Dickson and Lamb² and by Rodewald³. First, the great boosting of northerlies during the winter months resulted in a steep decline in the mean winter air temperature throughout this sector, especially in those areas lying to the north and west of the atmospheric and oceanic polar fronts. South from the high arctic, towards the British Isles, the winter cooling becomes rapidly less marked but there is evidence that since the early 1960s the resulting decline in sea temperature on the European shelf during the critical spring months (March–May) has been sufficient to affect (favourably) the survival of North Sea cod, which in those latitudes are approaching the equatorward limits of their range⁴.

Apart from those effects, the strengthening of the northerly airstream has been responsible for a progressive southward extension of sea ice across the Greenland Sea, reaching a maximum extent in the spring of 1968. Icelandic observations⁵ suggest that this change took place in two stages. First, as the northerlies increased in strength, the hydrographic character of the cold east Greenland and east Icelandic currents was altered so that they became cooler and less saline as the proportion of polar water increased. The east Icelandic current, which had been an ice-free arctic current during 1948–63, became a polar current in 1964–71, transporting and preserving drift ice.

Ancillary biological changes have been attributed to this climatic deterioration. They include an economically important alteration of the annual feeding migration of the Atlanto–Scandian herring stock to Iceland^{2,6} and a delay in the spring production of phytoplankton in the southern Norwegian Sea (ref. 2, and G. A. Robinson, unpublished); a two-week reduction in the length of the growing season in England has also been reported during this period of cooling⁷.

In the winter of 1970–71, the striking climatic trends evident over the Norwegian–Greenland Seas came to an abrupt halt. The high-pressure anomaly cell over Greenland, which had for so long dominated the patterns of climatic behaviour in

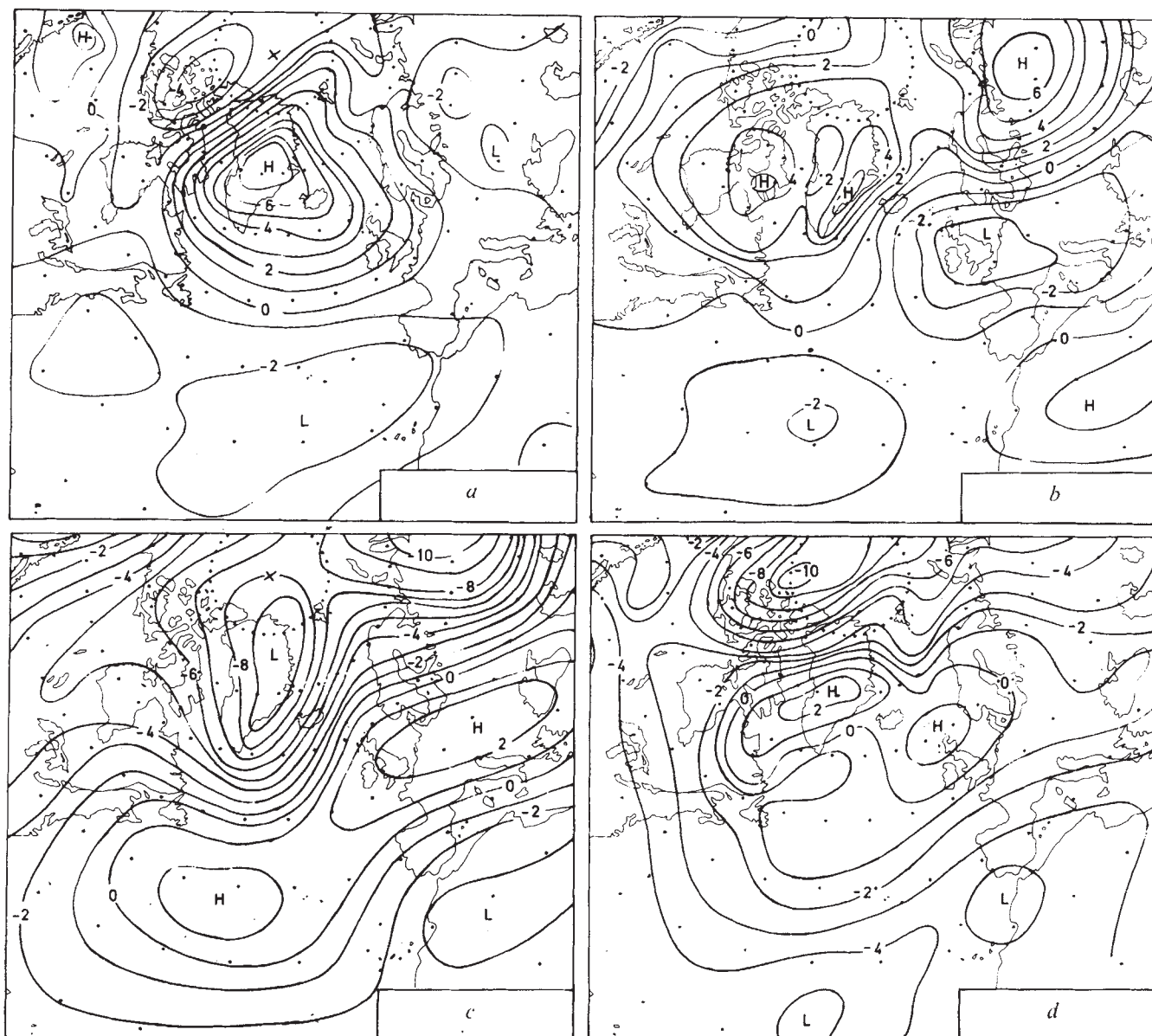


Fig. 1 Change of mean winter sealevel pressure (mbar) between the periods: *a*, 1900-39 and 1956-65; *b*, 1956-65 and 1966-70; *c*, 1966-70 and 1971-74; *d*, 1900-39 and 1971-74.

this sector, collapsed almost totally, and the mean pressure at sea level during winter fell by 9.6 mbar over Greenland between 1966-70 and 1971-74 (Fig. 1c). This ridge has not yet been totally eradicated, since in the earlier of the two periods the Greenland ridge had attained a peak intensity of over 12 mbar above the long term 'normal' (1900-39). Nevertheless, even though there is still a residual, high-pressure anomaly of some 2.8 mbar over Greenland (Fig. 1d) it is clear that the northerlies have been drastically weakened in the European arctic and subarctic since the winter of 1970.

The reason why such a persistent feature as the Greenland ridge should become weakened so drastically remains obscure, though the effect of the weakening is readily apparent in the succession of mild winters experienced over most of Europe since 1970. It is now also evident that this change has already brought about some amelioration in the marine climate off North Iceland. Fig. 2 illustrates the deviations of temperature and salinity from the 1950-58 mean, using observations made in June. Though conditions have not reverted to those of the mild 'arctic current' period (1948-63), both the temperature and salinity of the near-surface layer have risen since 1971 compared with the values obtaining during the 'polar current'

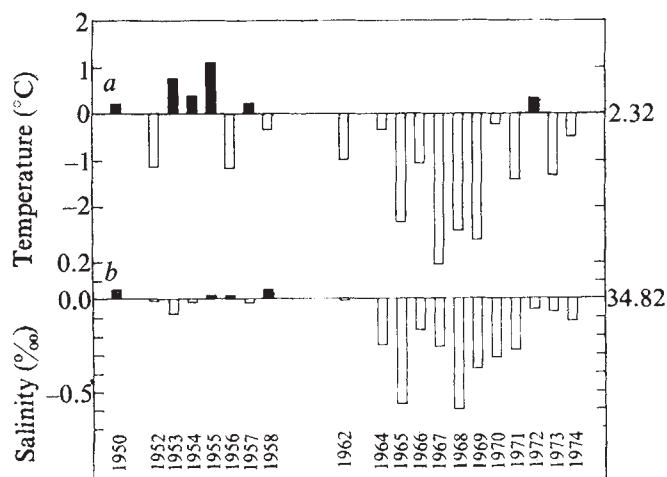


Fig. 2 Anomaly of temperature (*a*) and salinity (*b*) in June at a depth of 25 m in a study area between Iceland and Jan Mayen Island (67-69°N, 11-15°W; the averages for the normal period 1950-58 are shown).

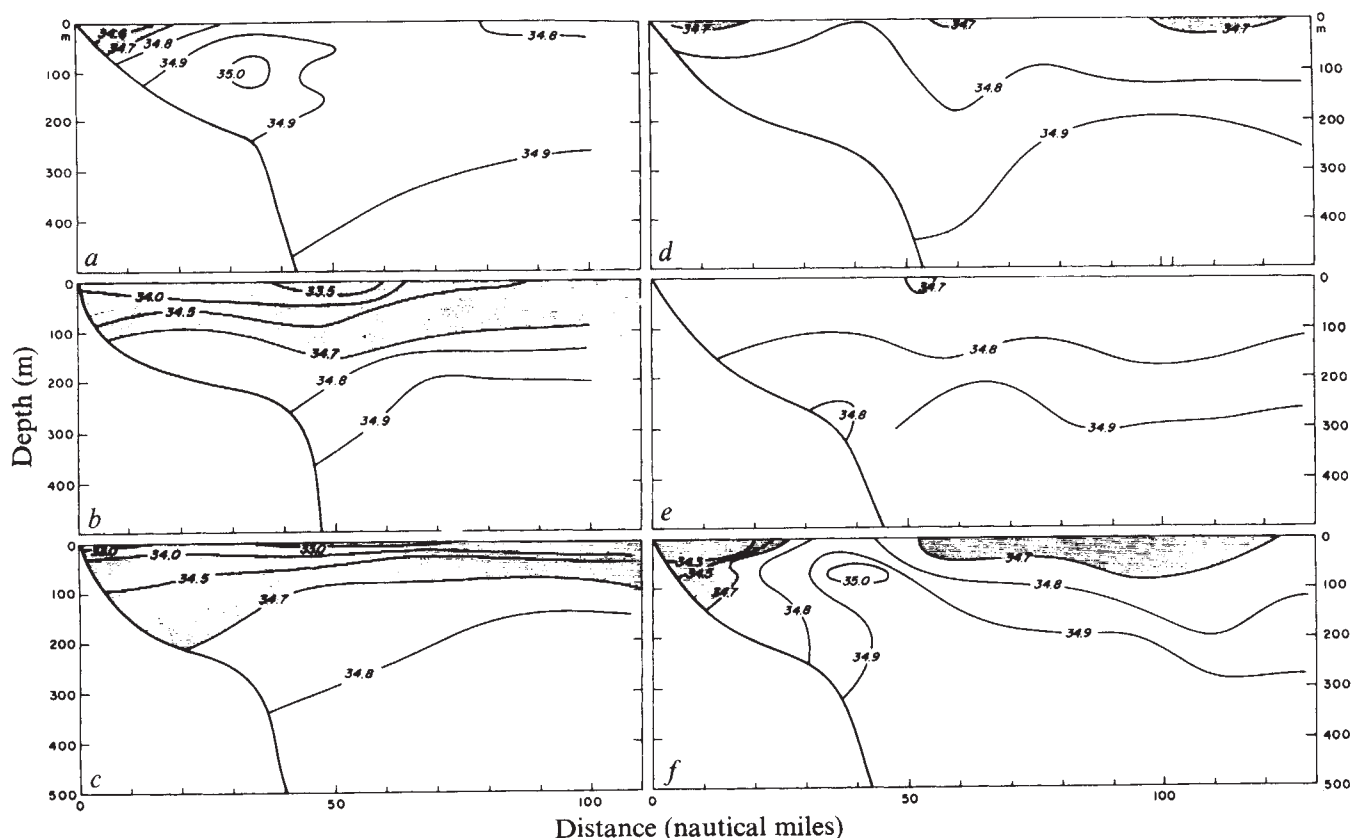


Fig. 3 Salinity distribution (‰) along a standard section worked north-eastwards from Langes (north-eastern Iceland) in the month of June: 1957 (a), 1965 (b), 1968 (c), 1972 (d), 1973 (e) and 1974 (f).

period between 1964 and 1971. The increase in surface salinity is of especial importance in this area since, at salinities of more than 34.7‰, before the freezing point is reached, the surface layers will attain a sufficiently high density to overturn and mix with the slightly warmer and more saline water at intermediate depths. At salinities of less than 34.7‰ the surface layers will not reach a sufficiently high density, even at the freezing point, to mix with these underlying layers and, instead, ice will tend to form.

Fig. 3 illustrates the recent weakening of polar influence off northern Iceland. The salinity section for June 1957 (Fig. 3a) is representative of a normal year during the relatively warm arctic period; 1965 (Fig. 3b) and 1968 (Fig. 3c) reflect conditions during the cold polar period (which reached its most extreme development in 1968) and the profiles for 1972–74 (Fig. 3d–f) represent conditions during the relatively mild period of the most recent years. As shown, polar water with a salinity of less than 34.7‰ was almost completely absent from the section in June 1972 and 1973, and though some renewed increase of polar water was observed in June 1974 its distribution was more restricted than in the 'polar current' period, 1964–71. Significantly, sea ice has posed no problem to navigation in northern Icelandic coastal waters since 1971 (H. Sigtryggsson, personal communication).

In general, it may be concluded⁸ that during the spring months of 1972–74 the Atlantic influx into northern Icelandic waters was stronger than in any year during the period 1965–71 (though remaining slightly weaker than the earlier 1950–60 normal⁹). The polar water component to the north and north-east of Iceland was weaker in the spring of 1972–74 than in any year since 1963 and only slightly stronger than in the warm period of earlier years. Thus the 'little ice age' observed in northern Icelandic waters in recent years seems to have ended with an amelioration of the marine climate during 1972–74. (It should be stressed, however, that this conclusion is diagnostic rather than prognostic.)

Although these physical and economic repercussions were most conspicuous close to the oceanic polar front at northern Iceland, similar trends of hydrographic change were also observed throughout the Norwegian–Greenland Sea during this period¹⁰. On 14 standard hydrographic sections worked in June across the entire ice-free area of the Norwegian–Greenland Sea from the latitude of Spitsbergen to that of south-western Norway (~60°–80°N), a general cooling has been observed between the mid 1950s and the late 1960s, with a rapid reversion to positive temperature anomalies occurring in the early 1970s. These broad scale changes in ocean temperature are in parallel with the strengthening and weakening tendencies of the northerlies in this sector, and presumably reflect changes both in the heat loss from the sea to the atmosphere, and changes in the poleward transport of warm water by the principal Atlantic current branches.

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Americium 242m in nuclear test debris

DIAMOND *et al.*¹ discussed the production, in a thermonuclear explosion, of isobars of mass numbers 239 and greater by neutron reactions on uranium, followed by β decay; much the same arguments seem to apply to phenomena in plutonium devices. Both cases, viewed in this way, suggest that mass number 241 will be represented by both ^{241}Pu and ^{241}Am , mass number 242 only by ^{242}Pu , and mass number 243 only by ^{243}Am . Such considerations neglect, however, the fact that weapons-grade plutonium is always more or less contaminated by ^{241}Am , and that this nuclide has an appreciable cross section (100 barn for thermal neutrons and slightly larger for epithermal neutrons) for neutron capture leading to ^{242}Am ; about 10% of such captures yield ^{242}mAm , the others going to the ground state. It seems, then, that one should expect to find in the debris from nuclear explosions not just two, but all three, of the long lived americium nuclides: ^{241}Am , ^{242}mAm and ^{243}Am .

Table 1 Concentrations in disintegrations per minute per 100 kg

	$^{239,240}\text{Pu}$	^{241}Am	^{242}Cm	^{244}Cm	$^{242}\text{Cm}/^{241}\text{Am}$
Sample A	216 ± 11	37.8 ± 0.7	0.21 ± 0.02	0.01 ± 0.004	0.54%
Sample B	18 ± 3	2.67 ± 0.06	0.013 ± 0.004	ND	0.49%

ND, Not detectable.

We have been led to this consideration by our finding of a small but readily measurable amount of ^{242}Cm in samples of seawater that had been contaminated by close-in fallout from an isolated, low yield nuclear test in 1962. The 60-l samples had been sealed in polyethylene containers from collection until being opened for radiochemical analysis in 1974. Americium 241 was separated from other nuclides, and prepared for α spectrometry, by radiochemical procedures^{2,3}; in this scheme curium isotopes accompany americium almost quantitatively. The americium plates from the samples in question were counted in a low-level α spectrometer for 8,000 min, in a special effort to detect any ^{244}Cm remaining from that produced in the test. Because the two samples represented different water depths, and thus different dilutions of the test debris, the concentrations of transuranic nuclides were very different, but the ratios among them were similar (Table 1).

α -Emissions of energy corresponding to those of possible naturally occurring contaminants were absent, confirming the cleanness of the radiochemical separation.

The ^{242}Cm cannot, after more than 25 half lives, represent curium originally in the samples; we believe there is little likelihood it represents laboratory contamination. Great care was taken in the radiochemistry, and we have never before seen ^{242}Cm in americium plates, except in samples expected to have this nuclide from waste disposal; also the uniformity of $^{242}\text{Cm}/^{241}\text{Am}$ ratio in two samples of different activity level argues against accidental contamination.

It seems to us necessary to conclude that the ^{242}Cm observed is the daughter of ^{242}mAm produced in the original nuclear event, probably by neutron capture of ^{241}Am . Direct observation of ^{242}mAm , at the concentrations indicated, would be quite impossible: detector backgrounds are too high for measurement either of its own X-ray emission, or of the β particles from its daughter ^{242}Am . Because detector backgrounds can be maintained, for solid state α spectrometry, at

extremely low levels, it should be practicable to measure ^{242}mAm in a variety of other samples, by searching for ^{242}Cm that is supported by a longer lived precursor. Actually we have remeasured one sample contaminated by fuel-reprocessing waste, and found that about 3% of the ^{242}Cm it contained was supported in this way.

The conclusion that ^{242}mAm has been produced, measurably, in nuclear tests, has some interesting consequences:

The ^{242}Cm daughter supported by decay of ^{242}mAm , in addition to being readily measurable in most areas of high fallout, should have, as a consequence of its generation by two rapidly successive radioactive decays (^{242}mAm decays, with a half life of 152 yr to ^{242}gAm , which decays with a half life of 16 h to ^{242}Cm), unusual environmental mobility, separating from other fallout transuranics such as ^{234}U has been found to separate from ^{238}U and ^{235}U .

Since ^{242}Cm decays to ^{238}Pu , its presence represents not merely an unevaluated additional source of this transuranic, but of a moiety of ^{238}Pu that would be expected to be more mobile than the fraction of that nuclide produced in the original event.

In plutonium the content of ^{241}Pu is greater in proportion to the fraction of Pu that has come as byproduct from electric power generation^{4,5}; ^{241}Pu will, however, be a less important constituent of breeder reactor plutonium⁶. These considerations suggest that americium 242m will be a significant component of the debris from future nuclear tests, or peaceful

nuclear explosions, although diminishing in proportion to the increasing use of breeder reactor plutonium.

We urge our colleagues in environmental radiochemistry to make a careful search for ^{242}mAm , both to establish baseline data describing its present distribution in nature, and to set limits on the influence of this nuclide on the environmental behaviour of its descendants, ^{242}Cm and ^{238}Pu .

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Tree remains in southern Pennine peats

RECENT work in Ireland¹⁻³, Wales⁴, and south-west England⁵ has demonstrated the coincidence in time of the onset of blanket peat accumulation with increased levels of prehistoric human activity in the vicinity; since many upland blanket bog areas in the British Isles have remains of trees embedded within the basal peat layers, it has been suggested that these tree remains represent a former forest cover, the deliberate clearance of which led directly to blanket peat formation. That is to say, the tree remains are assumed to immediately predate the oldest blanket peat layers.

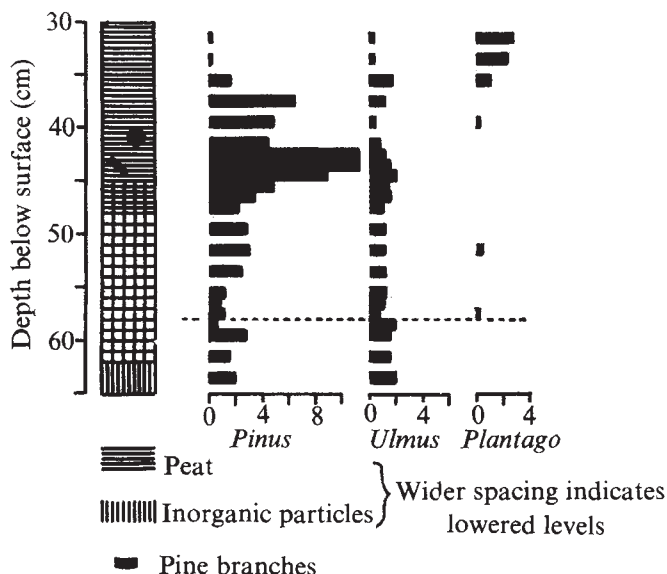


Fig. 1 Stratigraphy and selected pollen values for the basal peat layers at site 1, Lady Clough Moor, Yorkshire (at the blanket peat margin). The pollen values are expressed as percentage total land pollen. The broken line marks the position of the Elm Decline.

In the southern Pennines tree remains (in the form of twigs, charcoal, larger branches, procumbent trunks up to 2 m in length, or trunk bases and roots) occur widely within or below the basal blanket peat layers at altitudes from 290–595 m. Table 1 summarises the data currently available for 36 known sites, in terms of tree species recorded and the numbers of sites and altitudinal ranges at which they occur. The data are derived partly from records in the literature^{6–11}, partly from personal records, and particularly from extensive field surveys carried out by D. W. Yalden. The sites vary considerably in extent, ranging from those comprising the isolated tree stump in a moorland area of several hectares to those with locally dense concentrations of stumps and wood fragments. The majority of the sites are close to the margins of the blanket peat, but this distribution may merely reflect the relative scarcity of situations elsewhere where deep peat faces are exposed down to the underlying bedrock (and thus where

Table 1 Records of tree remains in southern Pennine blanket peats

Species	No. of sites	Altitudinal range (m)
Birch (<i>Betula</i> spp.)	16	320–595
Pine (<i>Pinus sylvestris</i>)	15	350–500
Oak (<i>Quercus</i> spp.)	10	320–470
Alder (<i>Alnus glutinosa</i>)*	5	290–400
Willow (<i>Salix</i> spp.)†	4	400–490
Hawthorn (<i>Crataegus</i> spp.)	1	about 350
Hazel (<i>Corylus avellana</i>)	1	about 320

* Includes 4 records of 'alder/birch wood' by Hicks¹¹.

† Includes one tentative record by Conway⁸.

basal tree remains can be observed). Tree remains are rarely recorded from altitudes above 500 m. Some sort of altitudinal zonation can be detected from the data in Table 1 for the four commonest species, with birch and pine remains predominant in the peat at the higher altitudes, and alder restricted to altitudes below 400 m. The widespread distribution of pine remains in the peat has not hitherto been recognised for the southern Pennines, and, indeed, pine remains have hitherto only been widely reported from Scottish blanket peats.

Several studies have shown that much of the blanket peat in the southern Pennines started to form in early Atlantic times, around 5000 BC; and accordingly it seems likely at first that these widespread pine remains represent relics of the pre-peat forests of Boreal times (as in Scotland¹²). But at the three sites so far investigated in detail, both pine and birch remains occur not at the soil–peat interface but actually within the basal peat layers. Although roots may extend downwards into the underlying mineral soil, the smaller twigs in particular are situated at distances of 5–20 cm upwards into the peat. Pollen diagrams from one of these sites (Lady Clough Moor, grid reference SK 105926, altitude 500 m) are shown in abbreviated form in Figs 1 and 2. At the peat margin on Lady Clough Moor, with peat depths of less than 1 m on slopes of up to 25°, pine remains are abundant in the peat, and coincide in position with a temporary peak of *Pinus* values in the pollen diagram (Fig. 1); farther back from the margin, on slopes of less than 10° and with deeper peat, birch remains are predominant, but the *Pinus* pollen peak is still present (Fig. 2). Clearly this *Pinus* peak can be referred to the Sub-Boreal period (pollen zone VIIb, about 3000–500 BC), since it occurs a short distance above the initial decline of *Ulmus* pollen values (the Elm Decline, shown

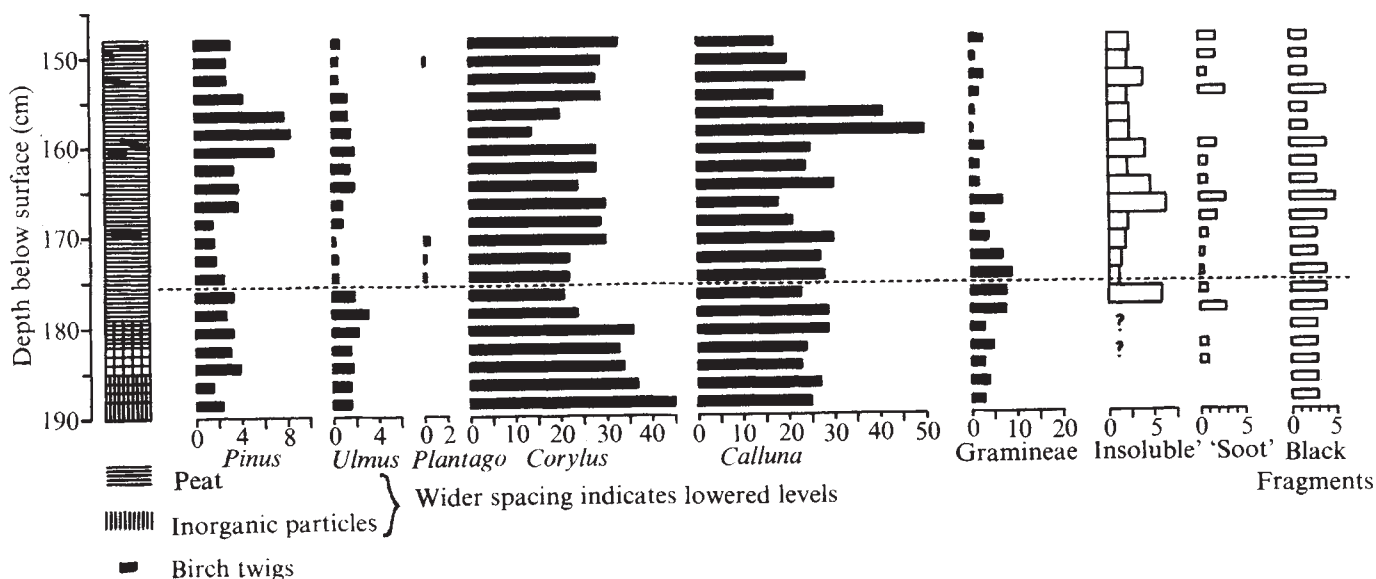


Fig. 2 Stratigraphy, selected pollen values, and estimates of carbon content for the basal peat layers at site 2, Lady Clough Moor, Yorkshire (30 m in from the blanket peat margin). The pollen values are expressed as percentage total land pollen. The broken line marks the position of the Elm Decline.

by a broken horizontal line in Figs 1 and 2) and the first appearance of occasional pollen grains of *Plantago*. Although more precise dating of the pine and birch remains is not yet available, it is possible that they could be as late as the uppermost alder/birch layers at Leash Fen¹¹, which have been radiocarbon dated at 1500–1790 BC. Without further extensive field surveys, pollen analyses, and radiocarbon datings it is clearly impossible to say whether this Lady Clough Moor site is typical, or whether some of the tree remains in the southern Pennines do indeed predate the inception of the peat blanket. There is no reason, however, to suppose that the site is not typical, and accordingly some mechanism has to be postulated whereby secondary colonisation of shallow blanket peat by trees became possible for a relatively short time during the Sub-Boreal period.

In the basal layers of all marginal peats so far investigated in the southern Pennines there is widespread evidence of burning—either in the form of microscopic carbon particles (similar to contemporary 'soot'), small charred plant fragments, or larger lumps of charcoal. Figure 2 shows some preliminary attempts to quantify this evidence of burning. The two right-hand columns show the abundance of soot-like particles and of charred plant fragments in the microscope slides prepared for pollen analysis, assessed subjectively on a five-point scale of frequency. The third column shows the weight of insoluble residue left after digestion of the peat with 20% potassium hydroxide and concentrated nitric acid (expressed as a percentage of the oven-dry weight of the peat). Under the microscope the majority of this insoluble residue seems to be carbonaceous. Carbonisation of the peat occurs in all samples examined, although there are clear differences in levels of carbon in different samples. Evidence of burning is least in the samples with high *Pinus* values, whereas high soot levels in the peat occur immediately above and below this *Pinus* pollen peak, and also immediately below the Elm Decline. Patterns of change in *Calluna*, *Corylus*, and Gramineae pollen values to some extent follow the evidence from burning patterns.

Probably the whole southern Pennine area is within the potential altitudinal range of tree growth, so that it is possible that before, and in the early stages of peat formation, colonisation by trees was prevented by recurrent fires. These recurrent fires could have been a natural phenomenon, but it is equally plausible that they were anthropogenic in origin. Numerous Mesolithic chipping sites, where flint or chert brought in from outside was worked into the characteristic artefacts, have been recorded from the southern Pennine uplands (see ref. 13 and references therein), and the area seems to have been a favoured seasonal hunting ground for Mesolithic populations for several millennia¹³. Most of these Mesolithic sites are clustered around the present-day blanket peat margins, at altitudes of 360–480 m. At two of these sites pollen analyses have shown considerable local disturbances in the vegetation at the level of Mesolithic occupation¹³. But the widespread carbonaceous remains in the basal peat layers indicate more than just local disturbance of the vegetation. There is evidence from contemporary hunter-gatherer communities of the widespread use of fire to increase the stocking capacity of hunting grounds and to control herd movements¹⁴, so that it is possible that sporadic burning was also carried out in the southern Pennine uplands before 3000 BC by Mesolithic hunters. Evidence of burning in the peats, however, continues upwards into the Sub-Boreal period, when Mesolithic populations had almost certainly been replaced by Neolithic cultures. Since at several sites in the southern Pennines there is apparent continuity of occupation from Mesolithic to Neolithic times¹⁵, it is possible that the uplands continued to be used primarily as seasonal hunting grounds for many centuries after the first advent of Neolithic peoples (at the time of the Elm Decline). With the gradual development of more intensive agriculture, anthropogenic pressure on the uplands may ultimately have diminished, and with reduced frequencies of

burning, trees were able to colonise the drier shallower peats. The subsequent disappearance of these trees could have been the result of natural death from waterlogging consequent on peat accumulation, but since most tree remains in the peat are of relatively young trees it is more probable that deliberate woodland clearance was involved. Contrary to recent hypotheses⁴ peat formation was already well advanced by this time.

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Regularities in duration of regional desert locust plagues

DURING a plague of desert locusts, *Schistocerca gregaria* (Forsk.), extensive areas are infested by successive generations of gregarious populations, and for working purposes a plague can be considered to be in progress as long as the progeny of swarms remain in swarms. In the periods between plagues, or recessions, the species exists mainly in low density populations, and such small swarms as occasionally occur seldom persist beyond one generation.

The total area invaded by desert locust swarms during plagues extends from West and North Africa eastwards to Assam, and from the Middle East to Tanzania, and can be divided into four major regions—western, eastern, north-central and south-central¹. Each region contains complementary seasonal breeding areas connected by migration circuits which are characteristic, but not always closed. From records assembled at the Anti-Locust Research Centre (now Centre for Overseas Pest Research) it was possible to reconstruct the incidence of regional plagues back to 1900 in the north-central and south-central regions, and to 1860 in the western and eastern regions. The data obtained were examined to see if there was evidence of plague regularities or of periodicities such as have been suggested for the eastern² and western³ regions. The main conclusions are summarised below; the full data and their discussion, and all the details of statistical analyses, are presented in refs 1 and 4.

Table 1 Frequency distribution of intervals between onsets of successive regional plagues

Interval (yr)	≤4	5–7	8–10	11–13	14–16	17–19
Frequency of occurrence	5	9	7	5	8	2

Parameters available for analyses were the lengths of regional plagues and recessions, and of the intervals between onsets of successive regional plagues. To have sufficient data for statistical tests, combined values from all regions were used in each analysis. This is not strictly valid, as swarms may move between regions, which are therefore not entirely independent. As the continuation of regional plagues seems to be independent of such move-

ments, however, we decided to pool the data and regard conclusions as tentative.

Table 1 shows that the intervals between onsets of successive plagues in the same region were very variable. The evidence of any regularity was not increased when plagues were considered in sequence, for in all the regions the serial correlation of successive intervals was not significant. As in three regions the correlations were negative, the intervals between onsets of one and the next but one plague were calculated; such double intervals were found, however, to vary from 9–28 yr. Thus the intervals provided no evidence of periodicity.

The frequency distributions of the durations of regional recessions and plagues (Fig. 1a and b) were considered separately. Both were tested by examining their goodness of fit to the frequency distributions which could be expected on the following hypothesis: once started, a plague or a recession continues independently of how long it has lasted, until a chance occurrence of conditions which bring it to an end. The respective χ^2 tests showed that the actual distribution of recession lengths was consistent with this hypothesis, whereas the distribution of the plague lengths was not, so that for them the hypothesis could be reasonably discarded.

It follows that the termination of a plague may depend on how long it has already lasted. When estimates of the probabilities of a plague finishing in a particular year were calculated from the frequency distribution of plague lengths they suggested (Table 2) that the probability of a plague lasting only 1 yr was quite high, but that of it

finishing after 2 yr was very small; thereafter the longer the plague lasted, the more likely it was to end, with the probability of it lasting more than 8 yr being very small indeed. In effect, only one regional plague lasted more than 8 yr and in 55% of cases they lasted 6–8 yr (Fig. 1b).

The overall lack of periodicity in the onsets of regional plagues arises from a combination of the lengths of the plagues, which show some regularities, with those of recessions, which do not. The large proportion of brief regional recessions (Fig. 1a) may partly be caused by their termination by swarm invasions from outside. But some of them came to an end as a result of local plague upsurges, suggesting residual populations capable of a rapid rise to plague level.

Table 2 Estimated probabilities of a plague finishing in a particular year when it existed at the beginning of that year

Plague existing in year:	1	2	3	4	5	6	7	8
Probability of it finishing	0.18	0.00	0.06	0.10	0.18	0.36	0.47	0.88

From the frequency distribution of regional plague lengths it seems that plagues may be of two types—longer ones, which seem to carry the seeds of their own destruction, and short ones. The termination of some recent plagues, both short and long, may have been contributed to by control measures. It is certain, however, that such measures used in earlier times could not have had any significant effect on swarming populations. It is also known that the ending of some of the plagues was associated with droughts^{1,6}. It seems doubtful, however, that climatic factors, acting either directly or by differing from those to which locusts may have been preconditioned by the photo-periodic regimes of their progenitors⁷, could have accounted for the termination of most of the longer plagues, which ended at comparatively regular intervals after their irregularly occurring onsets. A cumulative effect of parasites and predators could be consistent with such regular intervals, but a review of available data on effects of all biotic factors on this highly mobile species indicated that they probably did not materially affect large swarming populations⁸.

The tendency of the longer plagues to last a comparatively regular and seldom exceeded period suggests that their declines may be brought about by some deterioration in populations persisting in a crowded state for a long succession of generations. Deleterious changes in high density insect populations are known^{9–11} and in locusts the reproductive capacity becomes reduced on crowding¹². This suggestion could be tested, in the event of another desert locust plague, by comparing in standard optimum conditions the survival and reproductive potentials of members of swarming field populations differing by known numbers of gregarious generations from the initial swarms.

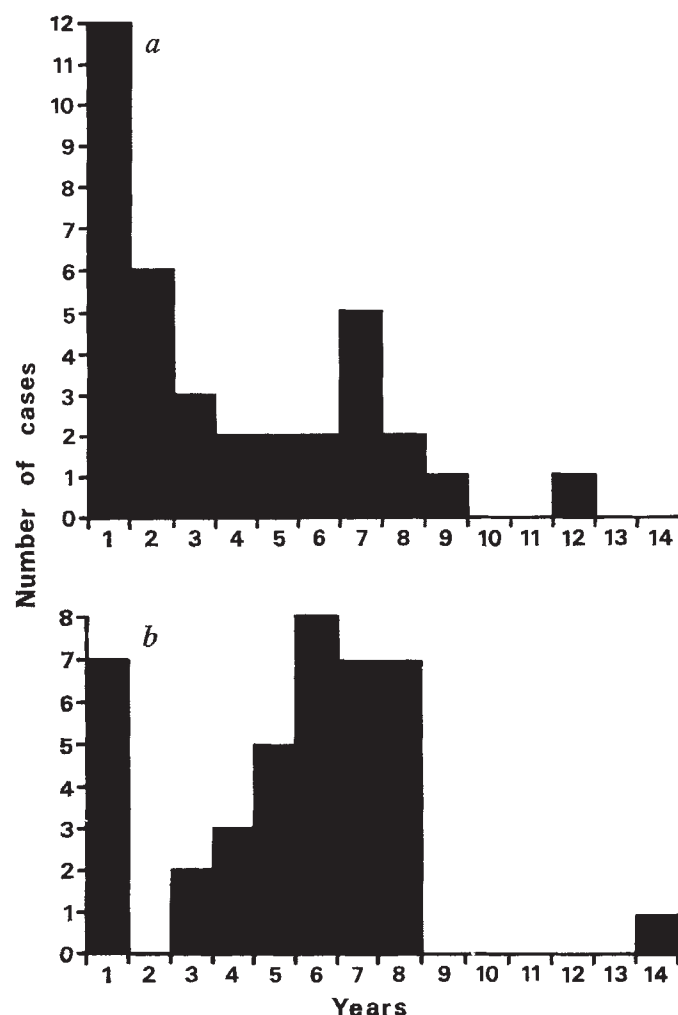
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Fig. 1 Frequency distribution of durations of regional desert locust recessions (a; $n = 36$) and plagues (b; $n = 40$).



Development of a desert locust plague

THE role of phase change in the development of plagues of the desert locust, *Schistocerca gregaria* (Forsk.), has been debated for many years¹⁻⁵. Much confusion has arisen because changes in behaviour, form and colour, which have all been used as indicators of phase status, do not proceed at the same rates. It is generally agreed, however, that behavioural changes are crucial in phase transformation. The subject at issue in the population dynamics of the desert locust is whether plagues, periods when extensive areas are infested by successive generations of gregarious populations, originate from an upsurge in numbers and associated transformation from the solitary-living to the gregarious state, or from successful breeding by gregarious populations postulated to survive continuously throughout the interplague recession periods³. Fundamental questions about the strategy of plague prevention depend on determining which of these hypotheses is correct^{1,3,4,6}.

An analysis of events preceding and during the 1968 desert locust plague has demonstrated the importance of phase transformation in plague development⁶. The upsurge of this plague, which began in mid-1967, was the result of an increase in numbers and densities of initially solitary-living locusts in successive generations. Each generation bred successfully following periods of rainfall over large parts of the normally arid recession area. It has been tentatively concluded from analysis of 36 occurrences of successful breeding that 25 mm is the threshold rainfall requirement for multiplication from mature parents to filial fledglings. This is slightly more than the 20 mm previously shown to be necessary for successful oviposition and incubation⁷. The timing of these falls of rain was such that a very short period usually elapsed between fledging and maturation, and this may be critical in upsurges.



Fig. 1 A sequence of breeding by desert locusts. The first generation (F_0) bred in early 1967, and the F_6 generation in late 1968. There is no evidence of breeding by F_7 adults. Arrows indicate direction of displacement between generations. Breeding areas are not drawn to scale.

Small and usually transient swarming populations are intermittently reported during recessions^{4,5}. Careful examination of all reports of desert locusts in 1966 and 1967 (ref. 6) suggests that the last two authentic swarms, before the buildup in mid-1967, were found in January 1966—one in Ethiopia and the other in Somalia. Neither produced gregariously-behaving adults in the next generation.

From mid-1967 to mid-1968 increasingly large numbers of locusts were reported from a number of places within the 14 million km² desert recession area stretching from West Africa to India. In each generation in each sequence of breeding there were progressive changes in the character of the populations, and the sizes of the infested areas. This can be illustrated by examining one such sequence, traced

over eight generations (Fig. 1). Detailed analysis has shown that the preliminary assessment of events outlined by Pedgley and Symmons⁸ was substantially correct. It is almost certain that the first generation bred in the southwestern interior of the Arabian peninsula in April–May 1967 (F_0). The second probably bred partly in that area and partly on the coastal plain of Yemen and Saudi Arabia in July 1967 (F_1). There is no evidence of gregarious behaviour in either of these generations, that is to say no bands of hoppers or swarms of adults were reported. One sequence that developed from this source involved a further generation breeding on the coastal plain after rain in November 1967, and two more on the coastal plain and in the interior of Saudi Arabia after rain in February and April 1968.

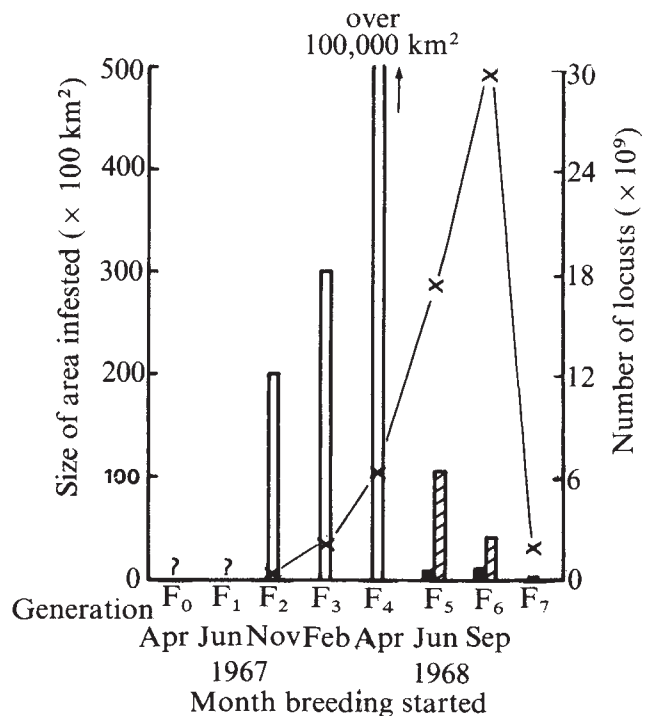


Fig. 2 The approximate numbers of locusts in each generation (x) and the gross areas infested by different types of population during the sequence of breeding shown in Fig. 1. Open bars show areas infested by locusts that were not fully gregariously-behaving; black, by swarms; striped, by bands. Gross infested area is the area needing treatment to reduce the population to an insignificant size.

In these three generations (F_2 , F_3 and F_4) an increasingly large number of locusts were found in an expanding area (Fig. 2) and an increasingly large proportion of the population showed some gregarious behaviour. The F_5 generation, however, was the first in which almost all the locusts were in bands and swarms. From May–August swarms of this generation emigrated from Saudi Arabia. About half migrated south-west across the Red Sea to Egypt and Sudan, where they started to breed in June 1968. Once gregarisation had occurred the gross infested area was reduced by about an order of magnitude between generations F_4 and F_5 in this sequence, although the number of locusts continued to increase (Fig. 2). Approximately half of the F_6 swarms emigrated from Sudan to Morocco, where low winter temperatures delayed maturation and almost all the swarms were killed before they could breed. Other swarms from Sudan migrated towards the Red Sea coastal plains; some were controlled and the remainder oviposited between September and November. There, the F_7 generation was much reduced by control, and the survivors died without producing gregarious locusts in the next generation. The progressive reduction in infested areas from F_5 to F_7 and in numbers from F_6 to F_7 (Fig. 2) resulted from control

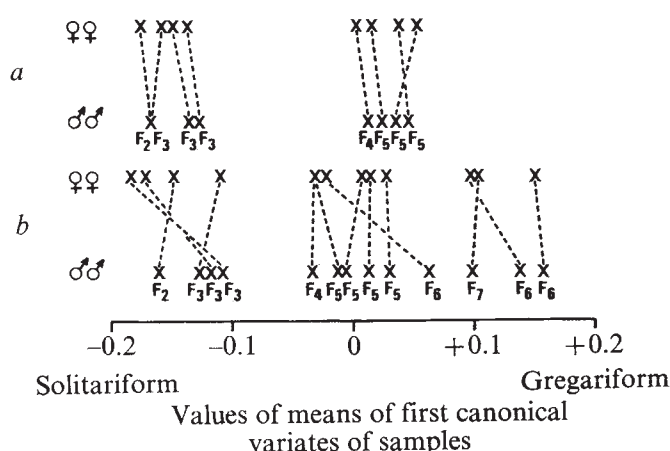


Fig. 3 Values of means of first canonical variates of samples from successive generations in two upsurge sequences. *a*, West Africa: F₂ Niger, September, 1967; F₃ Mali and Algeria, November 1967–March 1968; F₄ Mali, July 1968; F₅ Mali and Niger, September–November 1968. *b*, Sequence shown in Figs 1 and 2.

measures and natural mortality, and cannot be taken to represent typical development during a plague.

Morphometrics are available for several samples from generations F₂ to F₇ in the above sequence. The largest representative samples are shown in Fig. 3, which shows that the steady increase in gregarious behaviour was paralleled by a shift from the solitariform to the gregariform in the means of the first canonical variates⁹. The F₇ sample came from the Red Sea coast population that was the progeny of the most solitariform of the populations from which the F₆ samples were derived, so the shift towards gregariform continued in this generation. The two more gregariform F₆ samples were derived from the swarms which moved westwards from Sudan towards Morocco. Similar shifts can be seen in other sequences of breeding that contributed to the plague, the most complete of which is also shown in Fig. 3.

Phase transformation was progressive, and an integral part of the development of the most recent desert locust plague. One aspect of phase transformation is that during swarm formation (Fig. 2), the gross area requiring treatment contracts. The same area dosage rates are required for effective control of locusts whatever their density, so it is clearly more economical to concentrate control on the initial gregarious populations than to attempt to control the much larger areas infested at earlier stages in an upsurge, or the subsequent swarming generations which may be larger. A fuller account of this work will be published elsewhere.

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Seed-borne microorganisms stimulate seedcorn maggot egg laying

LARVAE of the seedcorn maggot, *Hylemya platura* (Meigen) may damage or kill young plants of many crop species by feeding on the cotyledons and plumules (Fig. 1). Damage may

be caused by larvae already present in the soil before the seeds are planted¹ or by larvae that hatch from eggs laid near germinating seeds^{2,3}. It has been assumed that the stimulus for oviposition is provided by the germinating seeds themselves but we suspected that seed-borne microorganisms growing on seed exudates could produce metabolites that promote oviposition. Many metabolic processes begin at the onset of seed germination, and concurrently organic substances are leached from the seeds which provide substrates for microorganisms. We now report that microorganisms growing on substrates from seeds produce metabolites which stimulate egg laying in *H. platura*, and that germinating microorganism-free seeds are not stimulatory. This is an excellent example of a complex ecological interaction between an insect pest, the host plant and organisms of the microenvironment.

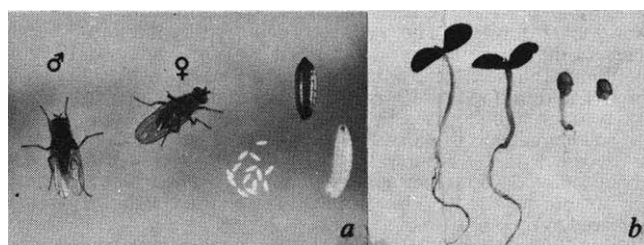


Fig. 1 *a*, Flies, eggs, puparium and larva of *H. platura*. *b*, Examples of varying degrees of injury on squash seedlings caused by earlier maggot feeding.

Squash (*Cucurbita pepo* L.) seeds, which elicit profuse oviposition³ were planted in a system designed to prevent microbial contamination and to provide a suitable ovipositional substrate. Seeds or their microorganisms were incubated for 24 h for complete seed imbibition and/or microbial growth to occur. Flies that had been reared using a modified Harris technique⁴ were then given access to beakers containing either the seeds or microorganisms for 24 h, immediately after which the eggs were counted⁵. All experiments were repeated five times.

We compared microorganism-infested seeds with seeds free from infestation. Earlier work demonstrated that Butternut squash seeds were preferred by the flies but we could not eliminate seed-borne microflora from this variety without killing the seeds. We therefore included Table Queen seeds whose microflora could be eliminated without impairing germination⁶. With both varieties, most egg laying occurred in beakers containing seeds infested with microorganisms,

Table 1 Oviposition by *H. platura* in beakers containing squash seeds incubated on water agar and covered by a layer of sterile acid-washed quartz sand

Seed	% Total eggs oviposited
Infested Butternut	47
Infested Table Queen	27
No seeds	17
Microorganism-free Table Queen	8

The results are all significantly different at the 5% level as determined by Duncan's multiple range test on arcsin transformations.

significantly fewer eggs were found in containers with no seeds, and very few with microbe-free seeds (Table 1). These results show that microorganisms on squash seeds stimulate *H. platura* oviposition. In addition, germinating squash seeds in the absence of microorganisms may deter oviposition (Table 1). Two additional tests not reported here gave similar results.

Before attempting to identify some of the microorganisms involved we had to develop a system enabling us to isolate them in pure culture. Since either germinating seeds or soil may provide the substrates for growth of the stimulatory microorganisms, we developed a system using squash seed exudate

Table 2 *H. platura* oviposition in beakers containing various media and microorganisms from squash seeds

Medium	% Total eggs oviposited*
SSEA† + microbes	49.8 (a)
SEA‡ + microbes	18.5 (b)
SEA	14.5 (b)
SSEA	8.9 (b)
Water agar	8.4 (b)

The microorganism inoculum was a mixture of microorganisms obtained from the Butternut seeds. The substrate was in polypropylene reservoirs surrounded by water agar and covered by a sterile glass-fibre filter and a layer of sterile acid-washed quartz sand.

*Percentages followed by different letters are significantly different at the 5% level as determined by Duncan's multiple range test on arcsin transformations.

†Squash seed exudate agar (SSEA) was prepared from a mixture of 100 g of Butternut squash seeds in 1 l of distilled water that had been shaken for 16 h. The seeds were removed by filtering through cheesecloth, the liquid portion clarified by centrifuging at 10,000g for 10 min followed by filtration through a Reeve-Angel 934 H glass filter. The resulting liquid was heated with sufficient agar to make a 2% solution and sterilised by heating at 120 °C for 20 min.

‡Soil extract was prepared by heating a mixture of 100 g of greenhouse soil with 1 l of tapwater at 100 °C for 30 min, then adding about 1 g of CaCO₃, filtering, and finally sterilising in the autoclave for 20 min at 120 °C. The complete soil extract agar (SEA) was prepared by mixing 100g of soil extract, 0.5 g K₂HPO₄, 1 g of glucose, 20 g of Bactoagar and 1 l of water. This mixture was heated until the agar was in solution and then sterilised at 120 °C for 20 min. The pH of SSEA was 4.9–5.1, that of SEA was adjusted to 8.4.

agar (SSEA) or soil extract agar (SEA) (Fig. 3). The media were inoculated with 200 µl of a mixture of microorganisms obtained by shaking 10 g of the highly stimulatory Butternut seeds for 16 h in 100 ml of distilled water at 23–25 °C; dilution plates of the mixture yielded 10¹⁰–10¹² microorganisms ml⁻¹ with Bacto-Nutrient Agar. When inoculated, heavy microbial growth occurred on both SSEA and SEA but the former was preferred for oviposition (Table 2). These results indicate that squash seed exudates provide substrates for microorganisms which produce stimulatory metabolites and provide a system for routine assay.

Table 3 Oviposition by *H. platura* in beakers containing various microorganisms grown on SSEA

Microorganism	% Total eggs oviposited*
<i>Pseudomonas</i> + <i>Torulopsis aeria</i>	47 (a)
<i>Pseudomonas</i> sp.	26 (b)
<i>Torulopsis aeria</i>	22 (b)
None	6 (c)

The substrate was in a polypropylene reservoir surrounded by water agar and covered by a sterile glass-fibre filter and a layer of sterile acid-washed quartz sand.

*Percentages followed by different letters are significantly different at the 5% level as determined by Duncan's multiple range test on arcsin transformations.

Among the pure cultures obtained from Butternut seeds, a bacterium, a *Pseudomonas* sp. (Migula)⁷ and a yeast, *Torulopsis aeria* (Saito) Lodder⁸, were the most effective elicitors of oviposition. Isolates of these organisms have been deposited in the USDA Northern Marketing Research Division Collection, Peoria, Illinois, and have been designated NRRL B-4287 and NRRL Y-7845, respectively. Mixed cultures of these two organisms stimulated oviposition to a greater extent (Table 3).

This work provides a model system for metabolite identification. Syntheses of ovipositional stimulants may improve population monitoring of *H. platura* in the field as is the case with pest monitoring and modelling using synthetic insect sex pheromones⁹.

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Defensive stoning by baboons

REPORTS of the use of tools in offence or defence by wild animals is limited to accounts of chimpanzees throwing branches at conspecifics, potential predators and at human observers¹. Anecdotal accounts of stone throwing by baboons² have been dismissed on the basis of the unreliability of correspondents and the improbability of oriented throwing by a quadruped anatomically incapable of overhand throwing³. In spite of several years of field study elsewhere in Africa, often in rocky terrain, there are no reports by professional field observers of deliberate stone throwing by baboons^{4–7}.

Nevertheless, in the course of a one-year study of three chacma baboon (*Papio ursinus*) troops living on the desert floor of the Kuiseb Canyon in South West Africa we observed numerous instances of stone release directed towards us.

Stoning by these baboons is done from the rocky walls of the canyon where they sleep and retreat when they are threatened by real or imagined predators. Stones are lifted with one hand and dropped over the side. The stone tumbles down the side of the cliff or falls directly to the canyon floor. We recorded the details of 23 such incidents involving the voluntary release of 124 stones towards us. All age and sex classes larger than 2-yr-olds participated at least occasionally in stoning (Table 1). But the most frequent participants are 4-yr-old and older males. The unhabituated nature of the troop members and the early stage of the study made individual identification impossible.

These stoning activities were accompanied by escape movements and typical "wahoo" alarm and barking calls, further identifying the context of the event as a predator-prey interaction. Such vocalisations are given typically in the presence of other predators such as leopards and sometimes hyaenas. The only animals preying on baboons in the Kuiseb Canyon today are the spotted hyaena (*Crocuta crocuta*), the brown hyaena (*Hyaena brunnea*) and man. Leopards lived in the canyon until recently but are now absent. Baboons have probably not been hunted by man in this canyon in recent years.

Stoning is aimed in the sense that the stone is released in such a way that it falls towards the observer, the stoning individual having moved to a position on the cliff directly above or opposite the observer. A stone is picked up, sometimes by prying it loose from the substrate, and released with an underhanded shovelling motion, or is simply dropped over the edge.

This frequently resulted in stones whizzing over our heads. Usually we could dodge; but occasionally two or more individuals release stones at approximately the same time, complicating evasion. Stoning persisted when we were far enough out from the walls to preclude any possibility we might be struck. On one occasion we saw a stone released in the typical manner when we were above the releasing individual.

Stoning was never observed in the context of intraspecific

encounters, either in intratroup combat or in intertroup encounters. Baboons remove stones from sleeping ledges when these sites are occupied at dusk. The material removed at such times is copious and gives the opportunity to evaluate the selectivity of defensive stoning. Of 200 randomly selected stones picked up from below a sleeping cliff following occupation of that cliff by one troop the mean size was 9 cm × 5 cm and weighed 88 g. The mean size of 22 stones released towards us from that cliff was 165 cm × 104 cm and weighed 583 g. Evidently baboons select relatively large stones to release towards intruders.

A special set of conditions is required to elicit this stoning behaviour. The initial response by baboons to our presence in the Kuiseb Canyon was to flee up the rocky slopes of the canyon wall. No stones are released in such circumstances, although occasionally one may be kicked free and tumble down the cliff. Late in the year of our study the troops became more accustomed to us and, although they retreated from our close approach, they dropped no stones. Stoning occurred only when a troop was partially habituated to our presence. This occurred in the middle months of our study and when we returned following a 2-month absence.

Table 1 Age/sex distribution of stoning individuals in three troops

Age/sex (No. of individuals, 3 troops)	No. of incidents	Expected incidents
I (11)	0	2.6
J1, J2 (10)	0	2.4
J3 (11)	5	2.6
J4M (2)	2	0.5
AF (21)	2	5.0
SAM (7)	3	1.7
AM (17)	7	4.1
Unknown	4	—

Expected values are derived from the age/sex distribution of the 79 individuals comprising the three troops observed.
I, Infant; J, juvenile; AF, adult female; SAM, subadult female; AM adult female.

For stoning to take place there must be associated cliffs with free or loose rocky material. Individuals initiating stoning generally persist with several stones. If loose material is exhausted the thrower may work vigorously, attempting to free loose rocky material from the canyon walls.

Stones were never seized when the relevant conditions prevailed and the troop was on a talus slope rather than on a steep cliff. The threat must come from beneath the cliff to elicit stoning. During some of our later observations of this behaviour we deliberately elicited stoning either to gain additional data or to demonstrate it to other observers. By carefully gauging the movement of the troop we were able to approach them when they were near a suitable cliff. Our presence moved them up on to it. By remaining beneath their cliff but out of range we were regularly able to elicit stoning behaviour. The need for troops to be partially habituated to begin stoning may explain the confusion concerning the reality of aimed stone throwing by baboons elsewhere in Africa. If stoning does occur elsewhere in Africa it could easily be missed. Animals habituated away from cliffs may not throw stones when observations are made in an otherwise appropriate environment.

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Eccentricity-specific dissociation of visual functions in patients with lesions of the central visual pathways

THE human retina projects not only to the lateral geniculate bodies and from there to the visual cortices, but in a parallel fashion to several other subcortical centres as well. One such centre is the superior colliculus, and it has been suggested that a rudimentary discrimination capacity of the locus of visual stimuli may be mediated by this structure¹. Results of anatomical studies with subhuman primates indicate that the macular region does not project to the superior colliculus^{2,3} (or that it is represented by a different population of retinal fibres which have not yet been identified by anatomical techniques⁴). The macular region seems to project only to the lateral geniculate bodies and from there to visual cortices, whereas the retinal periphery projects to several subcortical centres. This raises the question of whether a similar dissociation of projection between the central and peripheral retina also can be found in the human visual system. We present data obtained from patients with lesions of the central visual pathways which indeed suggest such a dissociation.

Besides kinetic and static perimetry⁵ the measurement of the critical flicker fusion (CFF) has proved to be a fruitful technique in the evaluation of visual dysfunctions in patients. A cortical lesion seems to result almost always in a diminution of the CFF in the contralateral visual field^{6,7}, also affecting the intact regions of the visual field and even the ipsilateral half field⁸; it has been suggested that the diminution of CFF in cases of brain injury is associated with changes in other visual functions such as the increment threshold⁹, by which retinal sensitivity is measured, although this claim does not seem fully supported by the published material.

Table 1 Effect of stimulus eccentricity on CFF (Hz)

Patient	Contralateral to		Intact side		Δ5°	Δ20°
	Lesion side 20°	5°	5°	20°		
E.A.	23.7	19.5	30.6	25.3	—11.1	—1.6
F.H.	31.0	26.0	33.3	29.0	—7.3	+2.0
G.J.	30.4	30.5	31.6	30.3	*—1.1	+0.1
Left side Right side						
Control subjects (n=9)	30.8 (23.5–39.1)	32.1 (25.5–36.9)	32.0 (27.6–36.9)	30.9 (23.0–40.8)	0.1	0.1

* $P \leq 0.01$

We measured the CFF in three patients with injuries of the central visual pathways and in nine control subjects. Two patients (see Table 1, E. A. and F. H.) had homonymous upper quadrant defects, whereas one patient (G. J.) had no obvious visual field defects, but had received a penetrating brain injury by a gunshot that ended in the lateral and caudal thalamic nuclei presumably injuring fibres of the geniculocortical pathway. The CFF in the patients and control subjects was measured at 5° and 20° eccentricity along the 225° (left lower quadrant) and 315° (right lower quadrant) meridians. These eccentricities were chosen because the anatomical evidence from primates suggests that the borderline of dissociation of central pro-

jection is beyond 5° but below 20° eccentricity³. For stimulus presentation the Tübinger perimeter⁵ was used. The CFF was measured binocularly after a careful monocular perimetry had been carried out. The diameter of the stimulus was 116 arc min visual angle, its luminance and the background luminance were 10 apostilb ($3,183 \text{ cd m}^{-2}$). The red fixation point had a diameter of 30 arc min visual angle. The fixation was observed by the experimenter throughout the CFF measurements. For determination of the CFF, the ascending method of limits was used, starting at a frequency well below the fusion threshold. With the exception of one patient (G.J.) seven measurements were taken at each eccentricity. The means of these measurements are shown in Table 1 for the patients E.A. and F.H. and in averaged form for the nine control subjects (the range is given in parentheses). The measurements on patient G.J. were much more extensive. On 7 different days 15 CFF values for 5° and 10 CFF values for 20° eccentricity were determined, each value being the average of 5 single measurements. The mean values of these 10 and 15 values are also shown in Table 1.

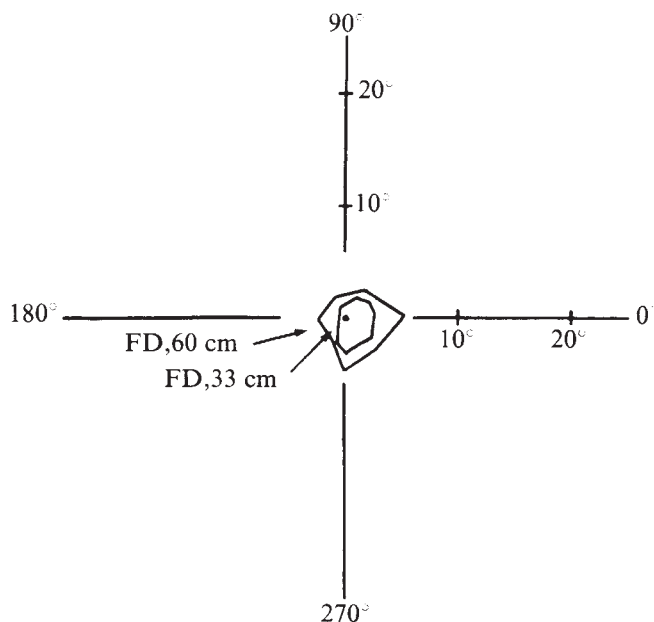


Fig. 1 Peephole visual field of a patient (H. H.) with a vascular lesion of both occipital lobes. S, Visual stimulus used to measure the visual field (116 arc min visual angle, 1,000 apostilb); BG, light intensity of the homogeneous background (10 apostilb); FP, fixation point (60 arc min visual angle, 1,000 apostilb); FD fixation distance.

The results (Table 1) show no difference of CFF for the control subjects if the measurements of 5° and 20° on both sides of the visual field are compared. As would be expected for these conditions, CFF is slightly lower towards the periphery¹⁰. For the patients E.A. and F.H. we observe a striking diminution of CFF contralateral to the lesion side for 5° eccentricity; CFF is 11.1 and 7.3 Hz less than contralateral to the intact side. Such a diminution of CFF is not seen for 20° eccentricity. For patient G.J. we observe no difference of CFF, if the measurements at 20° eccentricity on both sides of the visual field are compared (Wilcoxon test: $T=12$, $P \geq 0.05$). Although there is only a 1.1 Hz difference in CFF if the measurements at 5° eccentricity are compared, this difference turns out to be highly significant (Wilcoxon test: $T=1$, $P \leq 0.01$).

Our observations can be summarised as follows: a lesion of the central visual pathways results selectively in a diminution of CFF in the perifoveal area leaving CFF in the periphery of the visual field apparently unaltered. The diminution of CFF is observed in the intact part of the

visual field, and it may even be observed if no obvious visual field defect is present (patient G.J.) stressing the sensitivity of CFF for diagnostic purposes.

We suggest that the selective influence of a lesion of the central visual pathways on CFF in the perifoveal area but not in the periphery of the visual field is a consequence of a dissociation of retinal projection as it has been observed in lower primates. This is supported by a further observation in one patient (G.J.). Latencies of saccadic eye movements are much longer for targets at 5° eccentricity when they lie contralateral to the lesion side, but such a difference was not seen for targets with 20° eccentricity. An eccentricity-specific dissociation was not seen for increment threshold, however, which was higher at all eccentricities between 5° and 25° contralateral to the lesion side. The observation of a dissociation seems to be dependent on the selection of the test. Furthermore, the diminution of CFF at 5° , but not at 20° , suggests that the retino-collicular and cortico-collicular pathways are somehow involved in the perception of flicker. In experiments on programming of saccadic eye movements¹¹ it has been observed that targets within 10° of the visual axis are reached by one eye movement, whereas targets beyond 10° elicit at first an orienting response bringing the line of sight closer to the target, and then in a second step the target is reached by a corrective saccadic eye movement. These observations and the facts presented here favour the hypothesis that visual information is analysed in a qualitatively different manner by the nervous system according to its distance from the visual axis. The anatomical observations in subhuman primates would suggest that this differential analysis is dependent on a dissociation of the retinal projection in the human visual system.

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Evidence for visual function mediated by anomalous projection in goldfish

IN goldfish, there is a complete decussation of the optic nerves and only contralateral projection to the optic tectum is present. If one tectum is removed surgically, a few months later, the contralateral eye projects to the remaining ipsilateral tectum, superimposes the normal projection in a mirror-image fashion and retains its normal polarity¹. The method of mapping visuotectal projection involved presynaptic recordings of the optic fibre terminals in the tectum. It is not known, however, whether or not the anomalous projection is able to mediate visual behaviour. To determine whether the regenerated optic nerve fibres have formed functional connections in the tectum, post-synaptic recordings and (or) behavioural tests are required. We report here a preliminary finding that the anomalous ipsilateral

visuotectal projection, after removal of the contralateral tectum, may mediate the detection of a visual stimulus, with a threshold not significantly different from the normal projection. Sharma¹ has observed that in animals with complete removal of the right tectum "after removal of the right eye in these animals, the behaviour mediated by the left eye was apparently normal", and here we supplement those observations with psychophysical data.

Three adult fish were prepared as described previously¹; the entire left optic tectum was removed by cutting and suction, and the skull flap replaced; both eyes were left intact. Psychophysical testing began 125 d after tectal removal, at which time an organised ipsilateral projection is present. The fish was restrained in a transparent plastic envelope, and respiration recorded by suspending a thermistor, operated in its heating mode, in front of the fish's mouth. Breathing produced a temperature change in the thermistor; the resistance changes were recorded on a polygraph and were also converted to digital form with a saturating amplifier and sensitive relay. Details of the method are described elsewhere². The fish was suspended in an experimental tank with one eye facing a stimulus field (4 × 6 cm) at a distance of 45 cm. The field was illuminated constantly at a luminance of 3.3 cd m⁻². The stimulus consisted of five vertical bright bars, 15 mm across, which were moved back and forth twice each second at a rate of 11 cm s⁻¹.

Carbon rod electrodes were suspended on either side of the fish, through which an AC shock could be delivered; shock intensity was adjusted to the minimum level which would reliably produce a deceleration of respiration. Visual conditioned responses were established by the following procedure: (1) there was a 10 s period in which the number of breaths was counted and a stimulus was not presented; (2) this was followed by a 20-s delay; (3) a stimulus was turned on for 10 s and the number of breaths counted; (4) a shock of 250 ms duration was delivered on every trial when the stimulus was turned off. The criterion for conditioning (and detection of the stimulus) was as follows: total number of breaths during the 10-s stimulus was two less than during a similar period without a stimulus, and (or) there was at least one inter-breath interval greater than 1 s during the stimulus. To check for false-positive responses, a series of trials was run without a stimulus being presented during the second 10-s period but with electric shock being delivered as on stimulus trials; the incidence of response by the criterion of two-fewer breaths was less than 2%, and an inter-breath interval of more than 1 s was never recorded. Trials were presented randomly in time with an average interval of 3 min, to prevent temporal conditioning.

The number of breaths per 10-s interval varied between subjects and from day to day within a single subject, in the range 13–22. As an example of consistency of response, the mean breath rate for 1 d was 16.75 per 10 s (s.e. 0.38).

After reliable conditioned responses were established (which usually required only 8–10 trials), thresholds were determined by a staircase method: if a conditioned response was obtained to a given stimulus, the next stimulus was reduced in luminance by 0.3 log unit; if a response was not obtained, the next stimulus was increased by 0.3 log unit. This procedure continued until the reversals in the staircase reliably occurred in a small range of luminances.

All three fish produced vigorous conditioned responses with either eye facing the stimulus field. There was no reliable difference between the thresholds obtained with the two eyes. All measurements were made at least four times. The fact that thresholds were nearly identical with the two eyes argues against the possibility that responses with the right eye facing the stimulus were mediated by scattered and reflected light in the tank, which stimulated the left eye. This possibility was completely eliminated by removing the left eye, under anaesthesia, of one fish: there was no measurable change in threshold with the right eye facing the stimulus.

The visuotectal projection was mapped 148 d after tectal removal, and 4 d after the left eye was removed. The remaining

(right) eye projected on to the remaining ipsilateral (right) tectum in normal retinotopic order, as described previously¹. The anomalous ipsilateral visuotectal projection in goldfish is then able to mediate visual behaviour with sensitivity as great as the normal projection. The conditioned responses cannot be mediated by the remaining normal retinothalamic projection, as tectal lesions result in absolute scotomas³, although optokinetic nystagmus is still present⁴. Experiments are in progress to investigate visual acuity, summation area and visual direction in the reinnervated tectum.

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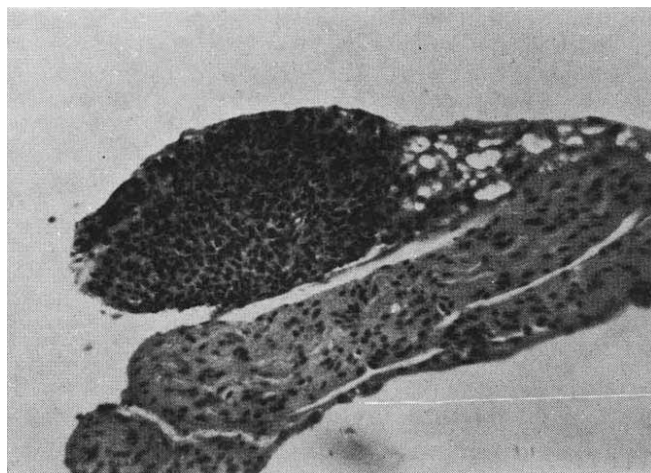
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Thymus rudiment of the athymic nude mouse

It is accepted that the polycystic organ, composed of branching ducts and differentiating during the first month of postnatal life into a system of interconnected cysts and acinar arrangements of serous and mucoid cells and containing almost no lymphoid cells, represents the dysgenetic thymus of the nude homozygote mouse^{1,2}. No epithelial cystic cells and no cells with specific inclusions which may be the cellular source of the thymic factors affecting pathways for differentiation of T lymphocytes, were found in the polycystic organ^{3,4}. The nude mouse has, however, not only the prethymic cells capable of differentiating into T cells in a thymic graft from an euthymic donor⁵, but also surprising numbers of θ -positive cells in the spleen⁶. It is suggested that these T cells may leak across the placenta from the euthymic mother into the nude foetus, may differentiate under the influence of the maternal thymic hormone, or may acquire their T-cell properties by some kind of non-thymic induction⁷.

Fig. 1 The lymphoepithelial rudiment of a *nu/nu* (BALB/c) male aged 122 d. H & E, ×160.



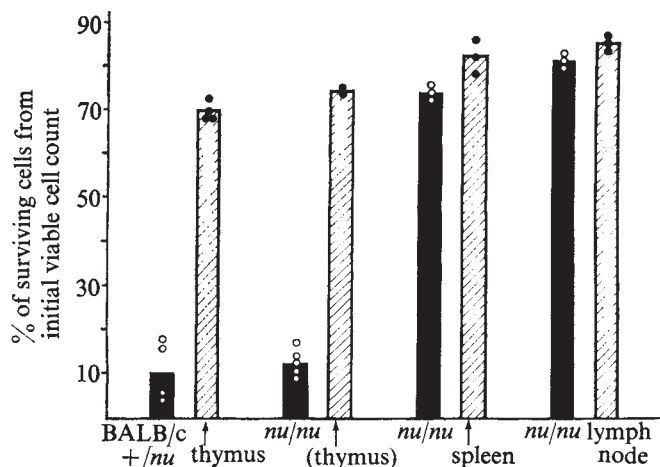
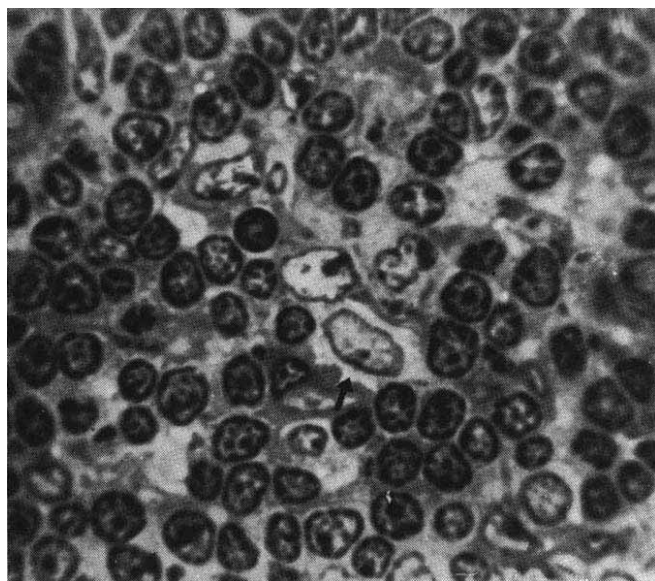


Fig. 2 Sensitivity of cells from euthymic mouse thymus and *nu/nu* lymphoepithelial rudiment (thymus?), spleen and lymph nodes to anti- θ antibodies. Solid columns, anti- θ -C3H+ complement; hatched columns, C only.

We have made histological serial sections of the entire area from the thyroid cartilage caudally to the bifurcation of the trachea in 42 *nu/nu* mice obtained from cross-intercross breeding of CBA mice with *nu/nu* mice, in 8 *nu/nu* mice from the eighth backcross to BALB/c mice (donated by Dr C. W. Friis) and in 3 *nu/nu* mice from the third backcross to C3H mice. All animals (both sexes, aged 30–122 d) were from *nu/nu* male $\times$ hybrid female matings. Between five hundred and two thousand haematoxylin-eosin stained sections of each mouse have been studied.

A paired ovoid structure (transversal sections usually not exceeding $400 \times 200 \mu\text{m}$ in diameter), composed of condensed lymphatic tissue and situated ventrally to the trachea and laterally to the polycystic organ was found in all *nu/nu* mice (Fig. 1). The structure was surrounded by a thin fibrous capsule and a poorly demarcated sinusoidal space underneath the capsule. On the medial aspect it was bordered with mature fat tissue. Typical small lymph nodes were found in the same sections laterally or dorsally to the trachea. In one *nu/nu* (CBA) male aged 4 months a single ovoid thymus-like organ with a dense cortical and loose medullary component and more pronounced subcapsular sinus was found in the same location as the lymphatic tissue described above.

Fig. 3 The lymphoepithelial rudiment of a *nu/nu* (C3H) female aged 88 d. Lymphocytes in the cortical part surrounding epithelial cells (arrow). Toluidine blue, $\times 1,520$.



This lymphatic rudiment was isolated from 12 *nu/nu* mice of all three genetic backgrounds and processed for electron microscopy (glutaraldehyde fixation, Vestopal embedding, semi-thin sections stained with toluidine blue, ultrathin sections contrasted with uranyl acetate and lead citrate observed in a Tesla BS 242 D electron microscope). Lymphoid cells teased from the rudiment of five *nu/nu* (BALB/c) mice were assayed with anti- θ C3H antibodies⁸. These were obtained from the ascitic fluid of AKR mice immunised repeatedly with C3H thymocytes and injected with S37 cells 14 d before killing. The *nu/nu* lymphoid cells were incubated with anti- θ C3H in Eagle's MEM for 30 min at 0 °C and subsequently with guinea pig complement (1:10) for 45 min at 37 °C. 2×10^5 – 4×10^5 cells from the rudiment were used and compared in the anti- θ assay with *nu/nu* spleen and lymph node cells and with BALB/c thymic cells. Control samples were incubated only in MEM and complement (30 min and 45 min). Each suspension was counted in a Bürker chamber and in the Trypan-blue exclusion test before and after incubation and the results expressed as a percentage of viable cells from the initial count.

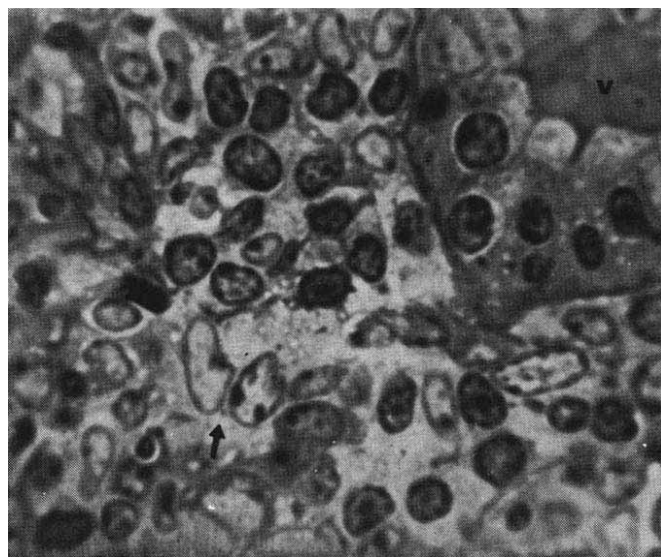


Fig. 4 The lymphoepithelial rudiment of a *nu/nu* (C3H) female aged 88 d. Lymphocytes and epithelial cells (arrow) in the central portion. A high endothelium venule (v) with lymphocytes in passage through the wall. Toluidine blue, $\times 1,400$.

Whereas few *nu/nu* spleen cells and even fewer lymph node cells were killed by anti- θ antibody and complement, the proportion killed of lymphoid cells from the *nu/nu* lymphatic rudiment and of BALB/c thymocytes was comparably high (80–90%), (Fig. 2). Absorption studies would be needed, however, before quantitative statements could be made about the average amount of θ antigen on the different cells examined.

In electron microscope and semi-thin sections the rudiment contained typical thymic lymphocytes (mostly of the dark variety) in nests and layers; among them mesenchymal reticulum cells, occasional macrophages, groups of epithelial cells with electron-dense granules, clear vesicles, lipid-containing vesicles and accumulated cysts were observed. The epithelial cystic cells resembled the thymic epithelial cystic cells. A few plasma cells and mast cells were also seen. The entire tissue was traversed with numerous blood capillaries and (in the deeper or central part) postcapillary venules with very high endothelium containing many lymphocytes in the passage from or into the lumen (Figs 3–5).

These observations indicate that *nu/nu* mice of different genetic background have a lymphatic rudiment containing θ -positive lymphocytes and secretory epithelial cells in the thymic region. It may be the organ responsible for the generation of T cells established in the *nu/nu* spleen. This organ

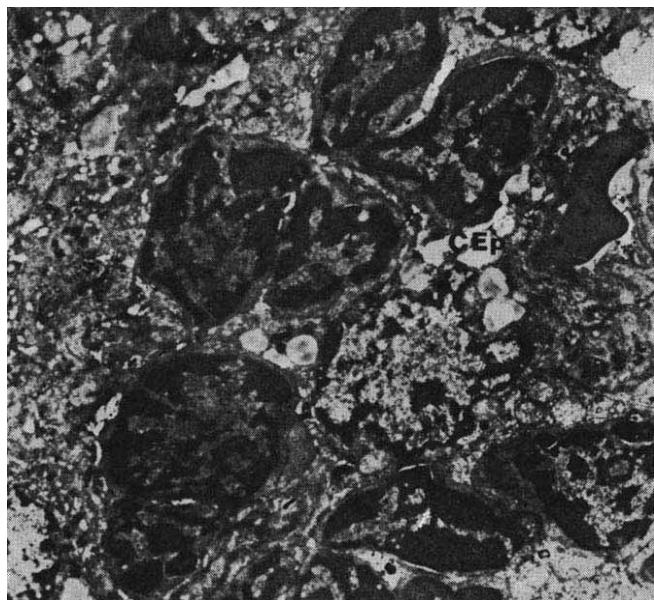


Fig. 5 Electron microscope appearance of the lymphoepithelial organ of a *nu/nu* (CBA) male aged 55 d. Lymphocytes surrounding a cystic epithelial cell (CEp). $\times 5,540$.

does not have all the histological attributes of a well-developed mammalian thymus; in its organisation it is more like the thymus of lower vertebrates. Ontogenetically, it may be a true derivative of the branchial pouch III entoderm, whereas the polycystic organ may represent the aberrant development of thymus IV only⁹, or of the ectodermal component (ductus ectobranhialis). The polycystic structures can be found also in *nu/+* hybrids with normal thymus development², and inside or outside the thymus of normal euthymic inbred mice (M.H., Vanecek, and P.R., unpublished).

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adult normal rats or mice⁷. These cultures at early stages consist of two main cell types, reticular and epithelial. In this communication we report the clonal differentiation of a third cell type, namely fully differentiated, striated muscle fibres in thymus reticulum cultures. This cell system, besides offering an approach to the analysis of muscle differentiation from adult mammalian precursors, has interesting biological and clinical implications.

The preparation of the cultures has been described in detail elsewhere⁷. Thymuses from young adult inbred Lewis rats (4–6 months) or C3HeB mice (4–6 weeks) were excised and carefully trimmed free of adjacent connective tissue as well as of the surface capsule. They were then minced with scissors, rinsed with phosphate-buffered saline (PBS), and subjected to differential trypsinisation to increase the concentration of the reticulum cells. The dissociated cells were suspended in Waymouth's medium enriched with 5% postnatal calf serum and plated into plastic Petri dishes (Fa. Greiner, Nürtingen), at a concentration of 30×10^6 cells ml^{-1} . In later experiments, we used gelatin-coated dishes⁸. After 2–3 d, most of the lymphoid cells were eliminated because of the relatively poor initial culture medium. The supernatant liquid containing the cellular debris was discarded and the cultures were replenished with Eagle's medium containing 15% horse serum. The medium was changed every 4 d.

During the first days of culture, single adhering thymus reticulum cells begin to divide and form confluent monolayers by day 7. In more than 95% of the cultures (about 150 independent platings) we found small spindle-shaped cells forming clones and fusing to contracting muscle fibres from day 8–10 (Fig. 1). These clones closely resemble those seen in myoblast cultures derived from chicken and rat embryos^{8,9}. To demonstrate myosin biosynthesis, duplicate cultures of varying ages were labelled with $3.75 \mu\text{Ci } ^{14}\text{C-leucine}$ ($342 \mu\text{Ci mmol}^{-1}$) per dish for 2 h in leucine-free minimal essential medium. Myosin was extracted and assayed as described elsewhere¹⁰. Table 1 demonstrates that concomitantly with muscle fibre fusion, myosin synthesis is initiated.

Table 1 Myosin biosynthesis by thymus muscle clones

	Days <i>in vitro</i>	Myosin ^{14}C (c.p.m. per 100-mm dish)
a	7	1,175 (background)
	7	1,476 (background)
b	12	25,457
	12	27,482

a, No fibre colonies present in the cultures; b, fibre colonies present in the cultures.

The origin of the thymus-derived striated muscles remains an open question. Trivially, these muscle cells could have developed from myoblasts in the connective tissue 'contaminating' the thymus reticulum cultures. To test this possibility, we removed not only the thymus capsules but also the adjacent cortical tissue and established parallel cultures from this material as well as from the rest of the thymus gland. The majority of the muscle clones arose from the latter starting material. Since the cortical fraction contains a higher proportion of connective tissue cells than the medullary fraction, it is probable that the muscle cells are derived from a genuine thymus component. This is corroborated by our failure to establish muscle cultures from non-thymic organs such as spleen, bone marrow, liver and kidney (H. W., unpublished).

Mammalian thymuses could contain committed myoblasts as physiological components. Indeed, myoid cells have been described in very early studies¹¹. Striated muscle was observed in reptile and avian thymus^{12,13}. The fact that there are but very few claims that myoid cells exist in normal adult mam-

Striated muscle fibres differentiate in monolayer cultures of adult thymus reticulum

THE thymus¹ is credited with a central role in the generation of clonal diversity of lymphocytes², and it may perform a critical function in the generation and/or prevention of autoimmune disorders³. Less attention has been paid to other, non-immunological thymus functions, such as control of haemopoiesis⁴, development of certain leukaemias⁵, and regulatory roles in the organism's hormonal balance⁶.

We have described a tissue culture method that allows growth *in vitro* of thymus reticulum monolayers derived from

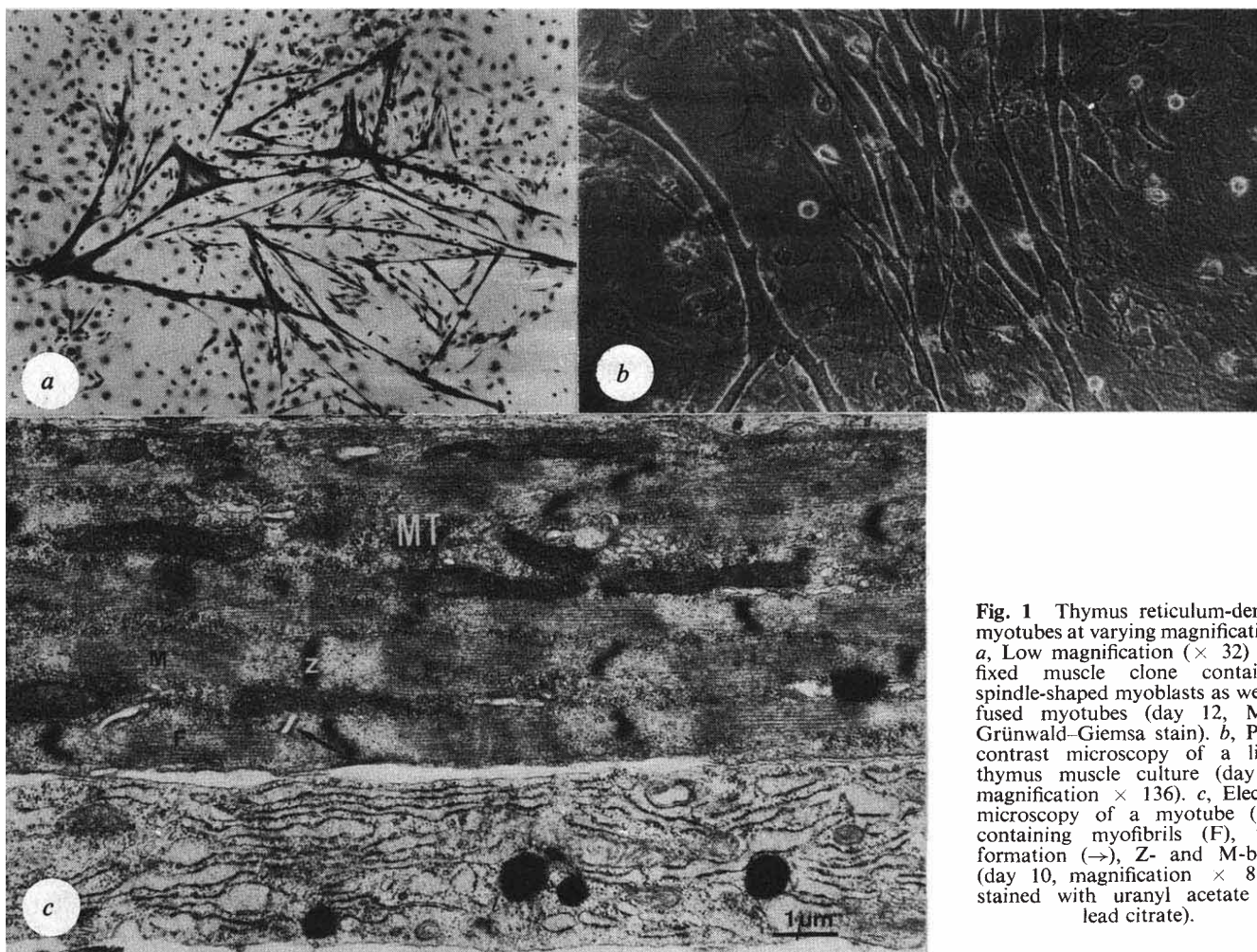


Fig. 1 Thymus reticulum-derived myotubes at varying magnifications. *a*, Low magnification ($\times 32$) of a fixed muscle clone containing spindle-shaped myoblasts as well as fused myotubes (day 12, May-Grünwald-Giemsa stain). *b*, Phase contrast microscopy of a living thymus muscle culture (day 10, magnification $\times 136$). *c*, Electron microscopy of a myotube (MT) containing myofibrils (F), triad formation ($\rightarrow$), Z- and M-bands (day 10, magnification $\times 8,925$, stained with uranyl acetate and lead citrate).

malian organs¹⁴ might argue against the possibility that thymus muscle clones are derived from pre-existing committed precursor cells.

Thus, we are left with the third possibility, namely, differentiation of thymus muscle clones from pluripotential stem cells. Several arguments seem to favour this possibility. It has been shown that, apart from muscle, tissues as different as osteocytes¹⁵ and chondrocytes (D. Yaffe, personal communication) can differentiate from thymus cells in particular conditions *in vitro*. In our cultures, the relatively long latency period of 8 d preceding muscle cell fusion could be due to differentiation processes of originally pluripotential stem cells. This is well in line with the strikingly similar behaviour of mouse teratoma cells. Undifferentiated OTT 6050 embryoid body cells differentiate into muscle tubes when they are cultured in very similar conditions to our thymus cells¹⁶.

The relationship of thymic cells and neoplastic pluripotential stem cells may be more than coincidental. It should be noted that the anterior mediastinal space shows the highest rate of teratomas, next to the gonads, and that these teratomas are thought to originate from thymic tissue¹⁷. It is tempting to speculate that mediastinal teratomas arise from neoplastic transformation of pluripotential stem cells in the thymus. Such stem cells, accumulated in this organ, may have a physiological role in the regeneration of the body's tissues. Experimental studies of congenital aplasias, for example in nude mice, should yield further information.

A final, very obvious implication could concern the correlation of thymic lesions and muscle autoimmune diseases like myasthenia gravis in particular. As we demonstrated before, thymocytes can be *in vitro* sensitised against autochthonous thymus reticulum cells³. It seems possible, therefore, that, following a defect of control, thymus lymphocytes could be

autosensitised against a thymus component bearing muscle antigens, and that such autosensitised lymphocytes cause the pathological lesions leading to myasthenia. Whether or not this is the case, our culture system provides the means to approach this problem experimentally.

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Continuous cultures of fused cells secreting antibody of predefined specificity

THE manufacture of predefined specific antibodies by means of permanent tissue culture cell lines is of general interest. There are at present a considerable number of permanent cultures of myeloma cells^{1,2} and screening procedures have been used to reveal antibody activity in some of them. This, however, is not a satisfactory source of monoclonal antibodies of predefined specificity. We describe here the derivation of a number of tissue culture cell lines which secrete anti-sheep red blood cell (SRBC) antibodies. The cell lines are made by fusion of a mouse myeloma and mouse spleen cells from an immunised donor. To understand the expression and interactions of the Ig chains from the parental lines, fusion experiments between two known mouse myeloma lines were carried out.

Each immunoglobulin chain results from the integrated expression of one of several *V* and *C* genes coding respectively for its variable and constant sections. Each cell expresses only one of the two possible alleles (allelic exclusion; reviewed in ref. 3). When two antibody-producing cells are fused, the products of both parental lines are expressed^{4,5}, and although the light and heavy chains of both parental lines are randomly joined, no evidence of scrambling of *V* and *C* sections is observed⁴. These results, obtained in an heterologous system involving cells of rat and mouse origin, have now been confirmed by fusing two myeloma cells of the same mouse strain,

The protein secreted (MOPC 21) is an IgG1 (κ) which has been fully sequenced^{7,8}. Equal numbers of cells from each parental line were fused using inactivated Sendai virus⁹ and samples containing 2×10^5 cells were grown in selective medium in separate dishes. Four out of ten dishes showed growth in selective medium and these were taken as independent hybrid lines, probably derived from single fusion events. The karyotype of the hybrid cells after 5 months in culture was just under the sum of the two parental lines (Table 1). Figure 1 shows the isoelectric focusing¹⁰ (IEF) pattern of the secreted products of different lines. The hybrid cells (samples *c-h* in Fig. 1) give a much more complex pattern than either parent (*a* and *b*) or a mixture of the parental lines (*m*). The important feature of the new pattern is the presence of extra bands (Fig. 1, arrows). These new bands, however, do not seem to be the result of differences in primary structure; this is indicated by the IEF pattern of the products after reduction to separate the heavy and light chains (Fig. 1*B*). The IEF pattern of chains of the hybrid clones (Fig. 1*B*, *g*) is equivalent to the sum of the IEF pattern (*a* and *b*) of chains of the parental clones with no evidence of extra products. We conclude that, as previously shown with interspecies hybrids^{4,5}, new Ig molecules are produced as a result of mixed association between heavy and light chains from the two parents. This process is intracellular as a mixed cell population does not give rise to such hybrid molecules (compare *m* and *g*, Fig. 1*A*). The individual cells must therefore be able to express both isotypes. This result shows that in hybrid cells the expression of one isotype and idiotype does not exclude the expression of another: both heavy chain

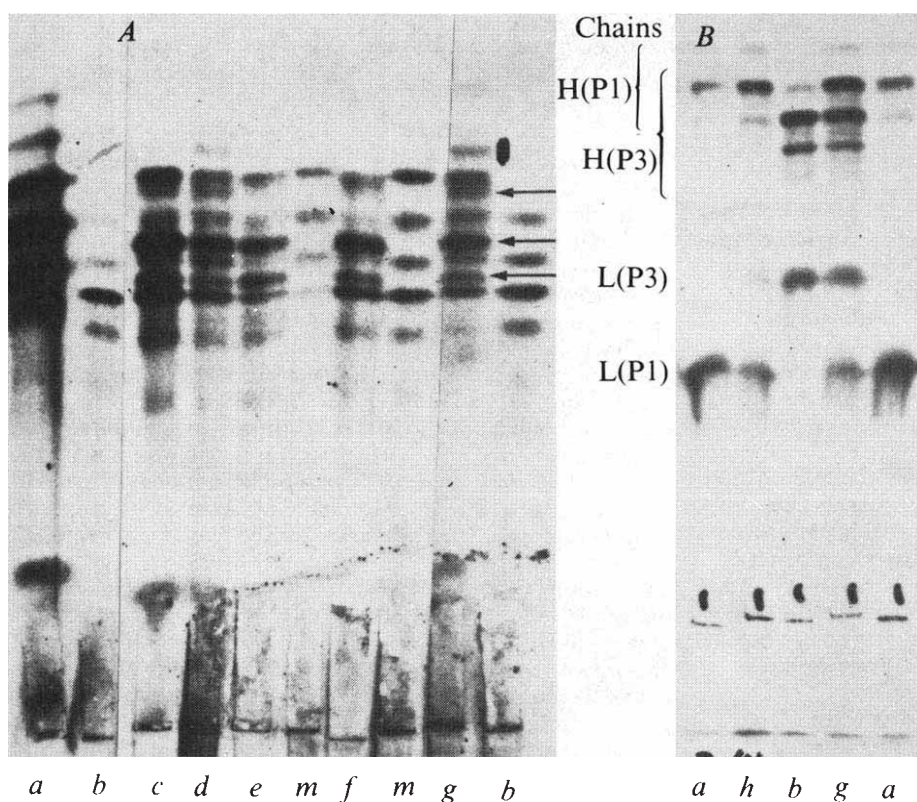


Fig. 1 Autoradiograph of labelled components secreted by the parental and hybrid cell lines analysed by IEF before (*A*) and after reduction (*B*). Cells were incubated in the presence of ¹⁴C-lysine¹¹ and the supernatant applied on polyacrylamide slabs. *A*, pH range 6.0 (bottom) to 8.0 (top) in 4 M urea. *B*, pH range 5.0 (bottom) to 9.0 (top) in 6 M urea; the supernatant was incubated for 20 min at 37 °C in the presence of 8 M urea, 1.5 M mercaptoethanol and 0.1 M potassium phosphate pH 8.0 before being applied to the right slab. Supernatants from parental cell lines in: *a*, P1Bul1; *b*, P3-X67Ag8; and *m*, mixture of equal number of P1Bul and P3-X67Ag8 cells. Supernatants from two independently derived hybrid lines are shown: *c-f*, four subclones from Hy-3; *g* and *h*, two subclones from Hy-B. Fusion was carried out^{12,9} using 10^6 cells of each parental line and 4,000 haemagglutination units inactivated Sendai virus (Searle). Cells were divided into ten equal samples and grown separately in selective medium (HAT medium, ref. 6). Medium was changed every 3 d. Successful hybrid lines were obtained in four of the cultures, and all gave similar IEF patterns. Hy-B and Hy-3 were further cloned in soft agar¹⁴. L, Light; H, heavy.

and provide the background for the derivation and understanding of antibody-secreting hybrid lines in which one of the parental cells is an antibody-producing spleen cell.

Two myeloma cell lines of BALB/c origin were used. P1Bul is resistant to 5-bromo-2'-deoxyuridine¹, does not grow in selective medium (HAT, ref. 6) and secretes a myeloma protein, Adj PC5, which is an IgG2A (κ), (ref. 1). Synthesis is not balanced and free light chains are also secreted. The second cell line, P3-X63Ag8, prepared from P3 cells², is resistant to $20 \mu\text{g ml}^{-1}$ 8-azaguanine and does not grow in HAT medium.

isotypes (γI and γ2a) and both *V_H* and both *V_L* regions (idiotypes) are expressed. There are no allotypic markers for the *C_K* region to provide direct proof for the expression of both parental *C_K* regions. But this is indicated by the phenotypic link between the *V* and *C* regions.

Figure 1*A* shows that clones derived from different hybridisation experiments and from subclones of one line are indistinguishable. This has also been observed in other experiments (data not shown). Variants were, however, found in a survey of 100 subclones. The difference is often associated with changes

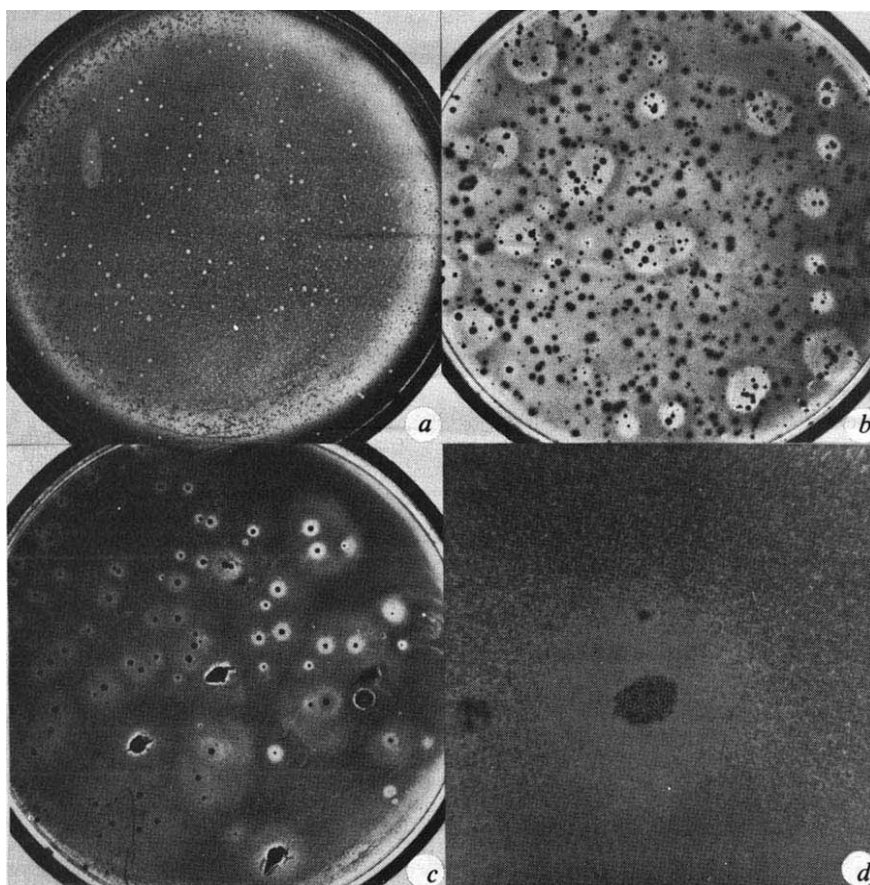


Fig. 2 Isolation of an anti-SRBC antibody-secreting cell clone. Activity was revealed by a halo of haemolysed SRBC. Direct plaques given by: *a*, 6,000 hybrid cells Sp-1; *b*, clones grown in soft agar from an inoculum of 2,000 Sp-1 cells; *c*, recloning of one of the positive clones Sp-1/7; *d*, higher magnification of a positive clone. Myeloma cells (10^7 P3-X67Ag8) were fused to 10^8 spleen cells from an immunised BALB/c mouse. Mice were immunised by intraperitoneal injection of 0.2 ml packed SRBC diluted 1:10, boosted after 1 month and the spleens collected 4 d later. After fusion, cells (Sp-1) were grown for 8 d in HAT medium, changed at 1–3 d intervals. Cells were then grown in Dulbecco modified Eagle's medium, supplemented for 2 weeks with hypoxanthine and thymidine. Forty days after fusion the presence of anti-SRBC activity was revealed as shown in *a*. The ratio of plaque forming cells/total number of hybrid cells was 1/30. This hybrid cell population was cloned in soft agar (50% cloning efficiency). A modified plaque assay was used to reveal positive clones shown in *b–d* as follows. When cell clones had reached a suitable size, they were overlaid in sterile conditions with 2 ml 0.6% agarose in phosphate-buffered saline containing 25 μ l packed SRBC and 0.2 ml fresh guinea pig serum (absorbed with SRBC) as source of complement. *b*, Taken after overnight incubation at 37°C. The ratio of positive/total number of clones was 1/33. A suitable positive clone was picked out and grown in suspension. This clone was called Sp-1/7, and was recloned as shown in *c*; over 90% of the clones gave positive lysis. A second experiment in which 10^6 P3-X67Ag8 cells were fused with 10^8 spleen cells was the source of a clone giving rise to indirect plaques (clone Sp-2/3-3). Indirect plaques were produced by the addition of 1:20 sheep anti-MOPC 21 antibody to the agarose overlay.

in the ratios of the different chains and occasionally with the total disappearance of one or other of the chains. Such events are best visualised on IEF analysis of the separated chains (for example, Fig. 1*h*, in which the heavy chain of P3 is no longer observed). The important point that no new chains are detected by IEF complements a previous study⁴ of a rat–mouse hybrid line in which scrambling of *V* and *C* regions from the light chains of rat and mouse was not observed. In this study, both light chains have identical *C_K* regions and therefore scrambled *V_L–C_L* molecules would be undetected. On the other hand, the heavy chains are of different subclasses and we expect scrambled *V_H–C_H* to be detectable by IEF. They were not observed in the clones studied and if they occur must do so at a lower frequency. We conclude that in syngeneic cell hybrids (as well as in interspecies cell hybrids) *V–C* integration is not the result of cytoplasmic events. Integration as a result of DNA translocation or rearrangement during transcription is also suggested by the presence of integrated mRNA molecules¹¹ and by the existence of defective heavy chains in which a deletion of *V* and *C* sections seems to take place in already committed cells¹².

The cell line P3-X63Ag8 described above dies when exposed to HAT medium. Spleen cells from an immunised mouse also die in growth medium. When both cells are fused by Sendai virus and the resulting mixture is grown in HAT medium, surviving clones can be observed to grow and become established after a few weeks. We have used SRBC as immunogen, which enabled us, after culturing the fused lines, to determine the presence of specific antibody-producing cells by a plaque assay technique¹³ (Fig. 2*a*). The hybrid cells were cloned in soft agar¹⁴ and clones producing antibody were easily detected by an overlay of SRBC and complement (Fig. 2*b*). Individual clones were isolated and shown to retain their phenotype as almost all the clones of the derived purified line are capable of lysing SRBC (Fig. 2*c*). The clones were visible to the naked eye (for example, Fig. 2*d*). Both direct and indirect plaque

assays¹³ have been used to detect specific clones and representative clones of both types have been characterised and studied.

The derived lines (Sp hybrids) are hybrid cell lines for the following reasons. They grow in selective medium. Their karyotype after 4 months in culture (Table 1) is a little smaller than the sum of the two parental lines but more than twice the chromosome number of normal BALB/c cells, indicating that the lines are not the result of fusion between spleen cells. In addition the lines contain a metacentric chromosome also present in the parental P3-X67Ag8. Finally, the secreted immunoglobulins contain MOPC 21 protein in addition to new, unknown components. The latter presumably represent the chains derived from the specific anti-SRBC antibody. Figure 3*A* shows the IEF pattern of the material secreted by two such Sp hybrid clones. The IEF bands derived from the parental P3 line are visible in the pattern of the hybrid cells, although obscured by the presence of a number of new bands. The pattern is very complex, but the complexity of hybrids of this type is likely to result from the random recombination of chains (see above, Fig. 1). Indeed, IEF patterns of the reduced material secreted by the spleen–P3 hybrid clones gave a simpler pattern of Ig chains. The heavy and light chains of the P3 parental line became prominent, and new bands were apparent.

The hybrid Sp-1 gave direct plaques and this suggested that it produces an IgM antibody. This is confirmed in Fig. 4 which shows the inhibition of SRBC lysis by a specific anti-IgM

Table 1 Number of chromosomes in parental and hybrid cell lines

Cell line	Number of chromosomes per cell	Mean
P3-X67Ag8	66,65,65,65,65	65
PIBu1	Ref. 4	55
Mouse spleen cells	—	40
Hy-B (P1–P3)	112,110,104,104,102	106
Sp-1/7-2	93,90,89,89,87	90
Sp-2/3-3	97,98,96,96,94,88	95

antibody. IEF techniques usually do not reveal 19S IgM molecules. IgM is therefore unlikely to be present in the unreduced sample *a* (Fig. 3B) but μ chains should contribute to the pattern obtained after reduction (sample *a*, Fig. 3A).

The above results show that cell fusion techniques are a powerful tool to produce specific antibody directed against a predetermined antigen. It further shows that it is possible to isolate hybrid lines producing different antibodies directed against the same antigen and carrying different effector functions (direct and indirect plaque).

The uncloned population of P3-spleen hybrid cells seems quite heterogeneous. Using suitable detection procedures it should be possible to isolate tissue culture cell lines making different classes of antibody. To facilitate our studies we have used a myeloma parental line which itself produced an Ig. Variants in which one of the parental chains is no longer expressed seem fairly common in the case of P1-P3 hybrids (Fig. 1*h*). Therefore selection of lines in which only the specific antibody chains are expressed seems reasonably simple. Alternatively, non-producing variants of myeloma lines could be used for fusion.

We used SRBC as antigen. Three different fusion experiments were successful in producing a large number of antibody-producing cells. Three weeks after the initial fusion, 33/1,086

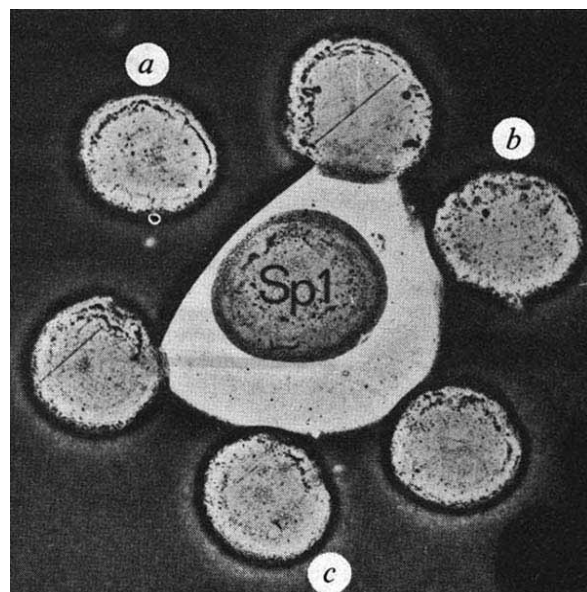


Fig. 4 Inhibition of haemolysis by antibody secreted by hybrid clone Sp-1/7-2. The reaction was in a 9-cm Petri dish with a layer of 5 ml 0.6% agarose in phosphate-buffered saline containing 1/80 (v/v) SRBC. Centre well contains 2.5 µl 20 times concentrated culture medium of clone Sp-1/7-2 and 2.5 µl mouse serum. *a*, Sheep specific anti-mouse macroglobulin (MOPC 104E, Dr Feinstein); *b*, sheep anti-MOPC 21 (P3) IgG1 absorbed with Adj PC-5; *c*, sheep anti-Adj PC-5 (IgG2a) absorbed with MOPC 21. After overnight incubation at room temperature the plate was developed with guinea pig serum diluted 1:10 in Dulbecco's medium without serum.

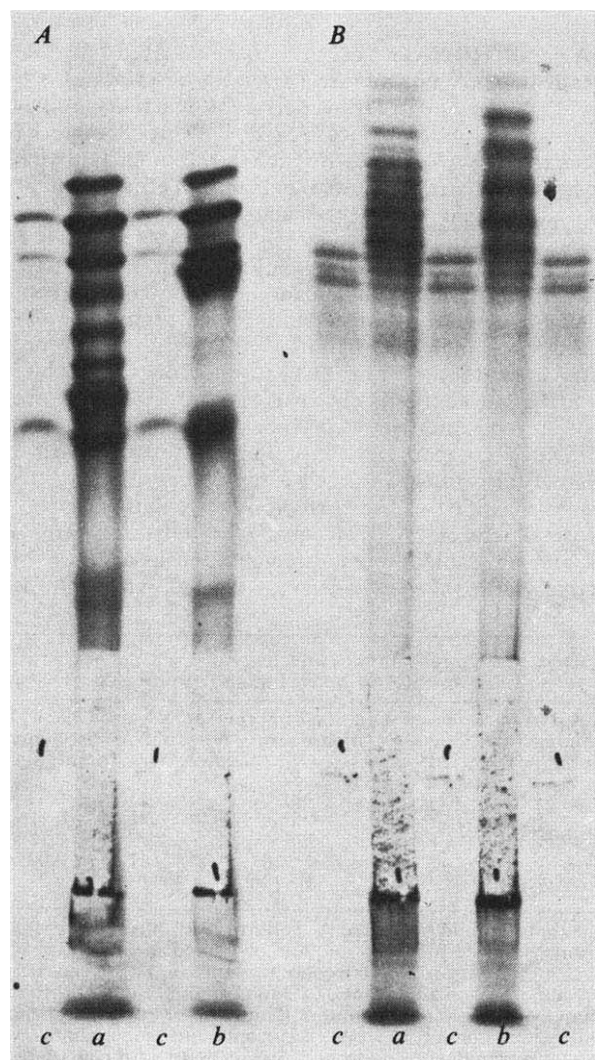


Fig. 3 Autoradiograph of labelled components secreted by anti-SRBC specific hybrid lines. Fractionation before (*B*) and after (*A*) reduction was by IEF. pH gradient was 5.0 (bottom) to 9.0 (top) in the presence of 6 M urea. Other conditions as in Fig. 1. Supernatants from: *a*, hybrid clone Sp-1/7-2; *b*, hybrid clone Sp-2/3-3; *c*, myeloma line P3-X67Ag8.

clones (3%) were positive by the direct plaque assay. The cloning efficiency in the experiment was 50%. In another experiment, however, the proportion of positive clones was considerably lower (about 0.2%). In a third experiment the hybrid population was studied by limiting dilution analysis. From 157 independent hybrids, as many as 15 had anti-SRBC activity. The proportion of positive over negative clones is remarkably high. It is possible that spleen cells which have been triggered during immunisation are particularly successful in giving rise to viable hybrids. It remains to be seen whether similar results can be obtained using other antigens.

The cells used in this study are all of BALB/c origin and the hybrid clones can be injected into BALB/c mice to produce solid tumours and serum having anti-SRBC activity. It is possible to hybridise antibody-producing cells from different origins^{4,5}. Such cells can be grown *in vitro* in massive cultures to provide specific antibody. Such cultures could be valuable for medical and industrial use.

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Naturally occurring cytotoxic tumour reactive antibodies directed against type C viral envelope antigens

TYPE C RNA viruses are frequently present in murine tumours, but the nature of the association between these viruses and malignancy is uncertain. The oncogene theory^{1,2} contends that type C viruses possess oncogenic information and that malignancy is the result of activation of this genetic information. Alternatively, the expression of type C viruses in a tumour may be a consequence, rather than a cause, of the neoplastic change and may be beneficial to the host by providing for potential immunological detection and eradication of neoplastic cells³. If type C viral antigens are targets for an immune surveillance mechanism, then normal animals would be expected to show evidence of immune sensitisation against viral antigens expressed on the surface of tumour cells. Here, we present evidence that naturally occurring antibodies (NOA) cytotoxic for a type C virus releasing teratoma-derived cell line are directed against viral antigens.

TerA is one of several cell lines derived from a spontaneous ovarian teratoma of a C3H/HeIcrf mouse. NB1 is a cell line derived from a neuroblastoma adrenal metastasis which was present in the mouse bearing the primary ovarian teratoma. Whereas electron microscopic examination of TerA cells revealed abundant budding type C virus particles, no budding type C viruses were observed in electron micrographs of NB1 cells. Supernatant fluid from

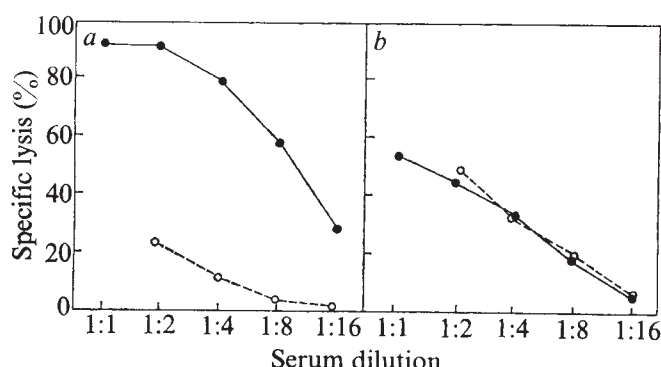


Fig. 1 Cytotoxic activity of normal serum of C3Hf/He mice unabsorbed (●) or absorbed with purified virus from TerA cells (○) and tested on either TerA (a) or NB1 cells (b). Virus was pelleted by ultracentrifugation and banded in a sucrose gradient using routine procedures. Briefly, supernatant fluid was collected 24 h after the cells were fed, was clarified by Millipore filtration (0.45 µm Millipore filter) and, after the addition of glycerin (6% final concentration), ultracentrifuged. Virus pellet was banded in a 20–60% linear sucrose gradient. It banded in a fraction with a density of 1.153 g ml⁻¹. This fraction was diluted with BSS and the virus reconcentrated by Amicon filtration (XM100 filter). Negative-staining electron microscopy revealed intact viruses with no discernible cellular debris. A 100 µl of volume of BSS containing an estimated 10¹⁰ sucrose-gradient-banded viruses from TerA cells was added to an equal volume of undiluted mouse serum. The mixture was incubated at 37 °C for 60 min and at 25 °C for 60 min. The tubes were centrifuged at 8,000g for 5 min and the supernatant serum tested for residual cytotoxic activity. Control sera were incubated with BSS. Cytotoxic activity was determined by a two-step RC'-dependent cytotoxicity assay as described previously^{6–8}. Normal mouse serum in the absence of RC' and RC' in the absence of normal mouse serum were not cytotoxic for either TerA or NB1 cells. The specific antibody-mediated lysis (%) was calculated by the formula:

$$\frac{(\% \text{ Cytotoxicity mouse serum} + \text{RC}') - (\text{RC}' \text{ control})}{\text{Maximum release} - \text{RC}' \text{ control}} \times 100$$

In all experiments TerA-derived virus specifically removed more than 80% of the cytotoxic activity of normal serum for TerA cells.

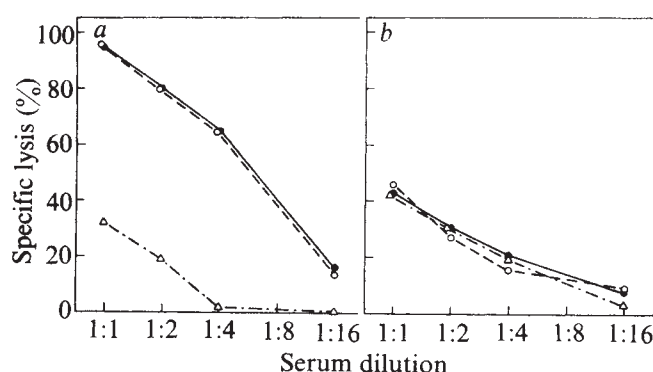


Fig. 2 Cytotoxic activity of normal mouse serum of C3Hf/He mice unabsorbed (●) or absorbed with either uninfected (○) or TerA virus-infected (△) SC1 cells, tested against TerA (a) or NB1 cells (b). Supernatants from TerA cell cultures were added to subconfluent cultures of SC1 cells. Cultures were treated as described in Table 1 and supernatants were assayed for reverse transcriptase as described previously⁴. Supernatant fluid from the infected SC1 cell lines was positive for reverse transcriptase activity (15 pmol ml⁻¹), whereas supernatant fluid from uninfected SC1 cell lines contained no reverse transcriptase activity. Undiluted normal mouse serum was absorbed for 40 min at 4 °C with a one-third volume of SC1 cells, either uninfected or infected 9 d previously with TerA-derived virus, and was tested for residual cytotoxicity against TerA and NB1 cells. Similar results have been obtained in two other experiments.

TerA and NB1 cell cultures was assayed for RNA-dependent DNA polymerase (reverse transcriptase) activity and DNA-dependent DNA polymerase activity using the conditions described by Ross *et al.*⁴. The pelletable RNA-dependent DNA polymerase activity (pmol ³H-thymidine monophosphate incorporated per ml supernatant fluid) in supernatant fluid from TerA cell cultures was 20.83. The reverse transcriptase activity in supernatant fluid from NB1 cell cultures was 0.02. Supernatants from these cell lines did not contain significant levels of DNA-dependent DNA polymerase activity. Host range studies on TerA-derived virus indicated that the virus grew readily on the mouse indicator cell line SC1 but not on the rabbit cell line SIRC. In addition, the virus showed a 100-fold preference for replication in embryo-derived cultures from NIH Swiss mice compared with similar cultures derived from BALB/c mice in the XC cell assay⁵. The virus can, therefore, be classified as an N-tropic endogenous virus⁵.

Normal sera of C3Hf/He mice were tested in neutralisation and in radioimmune precipitation assays for NOA reactive with the TerA-derived type C virus. A 1:10

Table 1 Neutralisation of infectivity of TerA-derived virus for SC1 by normal mouse serum

Dilution of supernatant containing TerA virus	Dilution of normal mouse serum	RNA-dependent DNA polymerase activity (pmol)
1:1	Nil	25.58 ± 0.42
1:10	Nil	21.24 ± 0.12
1:100	Nil	7.74 ± 0.91
1:1	1:10	1.44 ± 0.09
1:1	1:100	16.24 ± 1.08

Supernatant fluid from TerA cultures was filtered through a 0.45 µm Millipore filter. To aliquots of filtered medium was added normal mouse serum to final concentrations of 1:10 and 1:100. The medium was incubated at 37 °C for 1 h and refiltered through a 0.45 µm Millipore filter. Aliquots (2 ml) of untreated and mouse serum-treated undiluted TerA virus containing supernatants were added to subconfluent cultures of SC1 cells (2 × 10⁵ cells in 30 ml Falcon T flasks). In addition 2-ml aliquots of 1:10 and 1:100 dilution of TerA supernatant was added to SC1 cells, and the medium changed on day 3. Cells were subcultured on day 5 and the medium changed on day 8. Supernatant fluid was collected on day 9 and assayed for reverse transcriptase activity (pmol ³H-thymidine monophosphate incorporated per ml supernatant fluid) as described previously⁴.

dilution of normal mouse serum markedly reduced the infectivity of the virus for SC1 cells. Significant neutralisation was also observed at a 1:100 dilution of mouse serum (Table 1). A radioimmune precipitation assay, using goat anti-mouse immunoglobulin antisera to precipitate ³H-uridine-labelled virus bound to mouse immunoglobulin, confirmed that normal mouse sera contained NOA reactive with the type C virus (Table 2).

TerA and NB1 cells can be readily lysed in the presence of rabbit complement (RC') by NOA present in sera of C3Hf/He mice^{6,7}. The antigens recognised by NOA on TerA and NB1 cells are, however, distinct. To determine whether NOA reactive with TerA cells were reactive with the type C virus produced by TerA cells, normal serum of C3Hf/He mice was preincubated with sucrose gradient banded TerA-derived virus before testing for residual cytotoxicity against TerA and NB1 cells. As shown in Fig. 1, absorption with purified virus greatly reduced the cytotoxicity of normal mouse serum against TerA cells but

Table 2 Radioimmune precipitation of ³H-uridine labelled TerA-derived virus by normal mouse serum

Dilution of mouse serum	Radioactivity (%) bound by serum immunoglobulin
1:1	83.3 ± 3.4
1:5	62.6 ± 4.4
1:25	42.0 ± 5.0
1:125	2.1 ± 1.7

TerA-derived virus was labelled with ³H-uridine (New England Nuclear) by incubating TerA cells with 10 µCi ml⁻¹ isotope for 24 h. Supernatant fluid (10 ml) was collected and filtered through a 0.45 µm Millipore filter. The virus was pelleted by ultracentrifugation and resuspended to a 100 µl volume, containing approximately 10¹⁰ viral particles. A 10 µl volume of either undiluted or diluted mouse serum or of balanced salt solution (BSS) was added to 10 µl virus suspension and the mixture was incubated at 37 °C for 1 h and at 25 °C for 1 h. A volume (30 µl) of goat anti-mouse immunoglobulin antiserum (Dr R. Asofsky, NIH) was added to the tubes. A 10 µl volume of BSS was added to the tubes containing undiluted mouse serum and virus and 10 µl normal mouse serum was added to tubes originally containing either BSS or diluted mouse serum. A precipitate developed in each tube. After 2 h incubation at 25 °C the tubes were centrifuged at 1,000g for 10 min. A 20 µl aliquot from each tube was mixed with cold 10% TCA. Precipitates were collected on Millipore filters and assayed for radioactivity. Duplicate determinations were carried out on each serum dilution. Results are mean ± s.e. of values obtained in three separate experiments, and are expressed as a percentage reduction in supernatant radioactivity in tubes initially containing virus plus mouse serum compared with tubes initially containing virus plus BSS.

caused no significant reduction of anti-NB1 cytotoxicity. In some experiments, absorption of normal mouse serum with purified TerA-derived type C virus completely removed cytotoxicity against TerA cells. In addition, when normal mouse sera were absorbed with either uninfected SC1 cells or SC1 cells infected with TerA-derived virus and tested for residual cytotoxicity against TerA and NB1 cells, virus-infected SC1 cells specifically absorbed anti-TerA cytotoxicity (Fig. 2).

Our data indicate that NOA capable of binding intact type C virus released from TerA cells, and therefore presumably directed against viral envelope antigens (VEA), is cytotoxic, in the presence of RC', for TerA cells. This finding contrasts with a report in which cytotoxicity was not demonstrated with NOA, shown by radioimmune precipitation assay to bind VEA of the Gross virus released from a cell line of AKR mice⁹. These authors used a single step cytotoxicity assay, rather than the more sensitive two-step assay used here. In an immunoelectron microscopic study NOA were shown to react with both the cell surface of myeloma cells and with VEA of the type C virus released from the cells¹⁰. It could not be concluded, however, whether the cell surface antigen and the VEA recognised

by NOA were identical or whether the normal serum contained two populations of NOA recognising distinct antigens on the cell surface and on the viral envelope. Sera of a variety of mouse strains are known to possess NOA capable of neutralising the xenotropic virus released from chemically treated cells of BALB/c mice¹¹. These sera were not tested for reactivity with cell-surface antigens. Normal mouse sera contain NOA reactive with a wide variety of tumour cell lines^{8-12,13}. As partial expression of type C viral genetic information may occur, it cannot be excluded that NOA reactive with tumour cell lines which do not express type C viruses may not also be directed against a virally coded cell surface antigen. Although all tumour reactive NOA may not be directed against type C viral antigens, the findings reported in this paper are at least consistent with the notion that the selective expression of type C viral antigens by a tumour may assist in the immunological recognition of the tumour.

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Antigen formation in metal contact sensitivity

THERE have been reports of experimental induction of contact sensitivity to dinitrochlorobenzene (DNCB) and dinitrofluorobenzene by injection of dinitrophenol (DNP) conjugated to lymphoid cells¹⁻³. The DNP group is known to be capable of acting as a hapten but it is difficult to apply the term 'hapten' to the case of inorganic metals which act as contact sensitizers since antibodies with specificity for the metal ions have not been demonstrated. This is hardly surprising in view of the small size of some of the metals implicated as sensitizers, yet metal salts have been shown to produce stimulation of lymphocytes from individuals with metal contact sensitivity⁴⁻⁶. The following experiments were designed to determine whether lymph node cells pulsed with beryllium were capable of inducing beryllium contact sensitivity.

Guinea pig lymph node cells or peripheral blood erythrocytes were incubated with 1 ml 500 µM beryllium sulphosalicylate (BeSSA, trace labelled with ⁷Be) in saline for 1 h at 37 °C (ref. 6). Lymph node cell and erythrocyte concentrations were 2 × 10⁷ and 10 × 10⁷ cells ml⁻¹, respectively. Radioactivity was counted before and after washing three times with culture medium without added serum. This number of washes was chosen arbitrarily as radioactivity continued to be released from

the cells even after eight washes. The amount of BeSSA remaining with the cell suspension after three washes was found to vary with individual experiments from 3 to 30% of the original BeSSA added.

Beryllium-treated lymph node cells (4.0×10^7) in 4 ml medium were injected intraperitoneally into normal recipient guinea pigs. Erythrocytes (16.6 or 13.3×10^7) in 4 ml medium were used for injection.

Recipient guinea pigs were tested for contact sensitivity to beryllium 14 d later by painting the shaved back with 100 μ l 1% beryllium fluoride (BeF_2) in methyl cellosolve-water-Tween 80 (45:45:10). Reactions were recorded at 24 and 48 h on an arbitrary scale 0-3 (ref. 7). Animals were classified as contact sensitive when skin test readings were at least 0.5 at both 24 and 48 h and when at least one of these readings had a value of 1 or more.

It was then necessary to determine what proportion of guinea pigs which had been used as recipients were capable of being sensitised to BeF_2 since only a proportion of outbred animals are potential responders⁸. Recipient guinea pigs were therefore painted on the left ear on 3 successive days with 15% BeF_2 in Triton X-100⁹ and skin tested 14 d later with 1% BeF_2 .

Table 1 Induction of contact sensitivity to BeF_2 by intraperitoneal injection of lymphocytes pulsed with BeSSA

Dose of BeSSA injected	Carrier	Skin test reactivity to 1% BeF_2	Sensitisation by painting with 15% BeF_2
0.22-0.33 μ mol	4×10^7 lymphocytes (60-80% viable)	9/11	ND
0.32 μ mol	13.3×10^7 erythrocytes	0/8	6/8
0.30 μ mol	—	0/6	2/4 (2d)
0.03 μ mol	—	0/4	4/4
—	4×10^7 lymphocytes	0/3	3/3

Outbred Dunkin-Hartley guinea pigs were used as both donors and recipients. ND, Not determined.

Table 1 shows that allogeneic lymph node cells pulsed with BeSSA were capable of inducing contact sensitivity to BeF_2 in normal recipient guinea pigs. Animals injected with an erythrocyte suspension containing a similar amount of beryllium did not develop contact sensitivity although 6 out of 8 recipients were shown subsequently to be capable of being sensitised. Similarly, guinea pigs injected with 0.03 or 0.3 μ mol BeSSA in culture medium alone gave no reaction, suggesting that free BeSSA leaking from lymph node cells is unlikely to account for their efficacy in inducing sensitisation.

An attempt was made to induce beryllium contact sensitivity by injection of lymph node cells treated with BeSSA and killed by incubation with 1% sodium azide for 45 min at 37 °C. Cell viability after washing was less than 10%. Five allogeneic Dunkin-Hartley guinea pigs received 4×10^7 lymph node cells intraperitoneally but none became sensitised to BeF_2 .

Table 2 Induction of contact sensitivity to BeF_2 in Dunkin-Hartley guinea pigs by intraperitoneal injection of strain XIII lymphocytes pulsed with BeSSA

Recipients	Lymphocyte donors	Dose of BeSSA injected	Skin test reactivity to 1% BeF_2	Sensitisation by painting with 15% BeF_2
Dunkin-Hartley	Strain XIII	0.03-0.25 μ mol	4/11	7/11
Strain XIII	Dunkin-Hartley	0.04-0.31 μ mol	0/12	ND

ND, Not determined.

Table 2 shows results of experiments in which strain XIII guinea pigs, genetically unresponsive to beryllium by painting skin with BeF_2 (ref. 8), were used as donors or recipients for beryllium-treated cells. Strain XIII lymph node cells, pulsed with BeSSA, were found to be capable of inducing beryllium contact sensitivity in Dunkin-Hartley recipients. Strain XIII guinea pigs which had received Dunkin-Hartley lymph node cells showed no evidence of skin reactivity to BeF_2 .

It is of considerable interest that lymph node cells treated with the chelated beryllium complex, BeSSA, induced beryllium contact sensitivity since beryllium in a highly chelated form has been shown to be ineffective in eliciting a skin reaction⁹. The fact that allogeneic cells were capable of inducing sensitisation would argue that in this system the injected cells were contributing to the formation of immunogenic conjugates rather than becoming actively allergised *in vitro* as suggested by Polak and Macher³ using DNCB. The finding that strain XIII lymph node cells were also effective in inducing sensitivity would further support the contention that immunogen was being transferred. Negative results following injection of killed cells could be explained by an alteration of the antigen following azide treatment.

We interpret these experiments as demonstrating that antigen capable of inducing contact sensitivity to beryllium is formed when lymph node cells are pulsed *in vitro* with BeSSA. At present there is no information on whether this antigen is attached to the cells or is released from them as a complex. It is unlikely that BeSSA combining *in vivo* with tissues of the recipient will account for these results, since free BeSSA injected intraperitoneally failed to sensitise.

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Induced thermal resistance in HeLa cells

IN mammalian cells, hyperthermia causes temperature-dependent changes in cell growth parameters¹, reduces DNA and protein synthesis rates² and cell metabolism³, and leads to a loss of proliferative capacity^{4,5}. Harris⁶ has shown that temperature-resistant pig kidney cells can be obtained after multiple exposures to very high thermal doses (colony-forming ability reduced to 10^{-5} - 10^{-6} of controls). The purpose of our experiments was to determine whether a single hyperthermic treatment (44 °C for 1 h) could induce a state of thermotolerance in cells, and if so, what was the mode of origin of the thermal resistant cells.

HeLa cells (Flow Labs, Rockville, Maryland) were maintained in exponential growth at 37 °C in medium 199 supplemented with 10% foetal calf serum (both from Gibco). Their doubling time in these conditions was 23-24 h. Proliferative capacity, as measured by colony-forming ability *in vitro*, was used as a measure of survival. Thermal doses were administered to these exponentially growing cells by immersion of culture flasks in a temperature controlled water bath (temperature constant to ± 0.1 °C). As it was known that nutritional factors are important in determining cellular response to hyperthermia⁷, a standard treatment protocol was used with cells being treated in full medium 20 h after inoculation in the treatment flasks.

After thermal treatments, cells were removed from the flasks by trypsinisation, combined with any floating cells, diluted and plated for colony formation in 5 ml fresh medium.

Figure 1 shows the survival response of exponentially growing HeLa cells treated at 44 °C for increasing times up to 3.5 h. Survival decreases exponentially as a function of time at 44 °C. This result is similar to that already reported^{4,5,7}, except that no (or a very small) shoulder region is apparent on the single dose-survival curve. Figure 1 also shows that the survival response is exponential even when survival is reduced to less than 0.1%. Whereas previous authors^{4,5} have shown cell cycle-dependent fluctuations in thermal inactivation, this observation suggests that subpopulations have similar inactivation rates, as a resistant tail is not apparent as cell killing increases. Note that Westra and Dewey⁴ have shown that the difference in thermal response for CHO cells comparing sensitive mitotic and S-phase cells and resistant G₁ cells was primarily the result of a decrease in the shoulder of the survival curves, whereas the slopes of the exponential portion of the curves did not change. Thus, whereas the heterogeneous cultures reported here could contain subpopulations, some or all of which might have shoulders, a composite survival curve would also have a shoulder. This lack of a shoulder on the single dose-survival curve shown in Fig. 1 implies that these HeLa cells do not accumulate sublethal hyperthermic damage.

When two-dose experiments were used to test for the recovery of our HeLa cells from sublethal hyperthermic damage (Fig. 2a), cell survival increased with increasing incubation at 37 °C between two thermal treatments of 44 °C for 1 h. This result is similar to that previously reported for HeLa S-3 cells by Palzer and Heidelberger⁵ who interpreted their data to be recovery

Fig. 1 Thermal survival response of exponentially growing HeLa cells. ●, Survival response of cells treated with single exposures of 44 °C for increasing time; ○, response of cells treated at 44 °C for 1 h, returned to the 37 °C incubator for 2 h and given second graded doses of 44 °C for increasing times. Standard errors of survival determinations are as shown

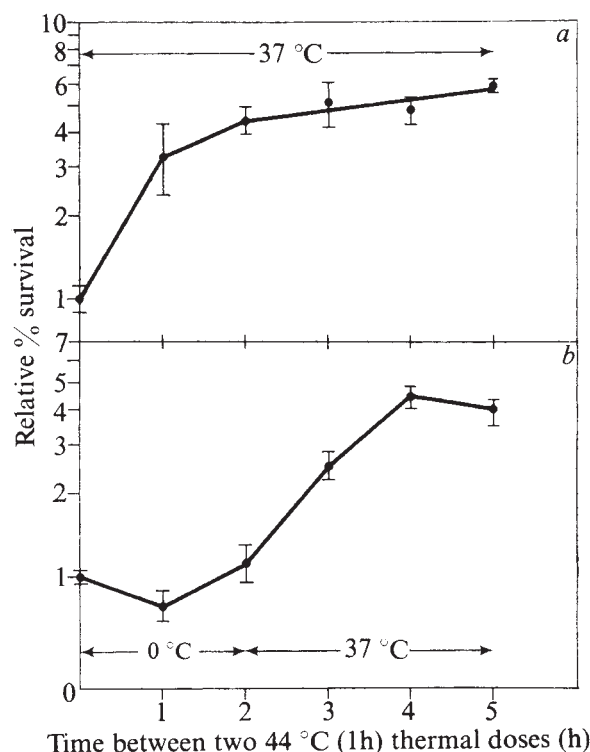
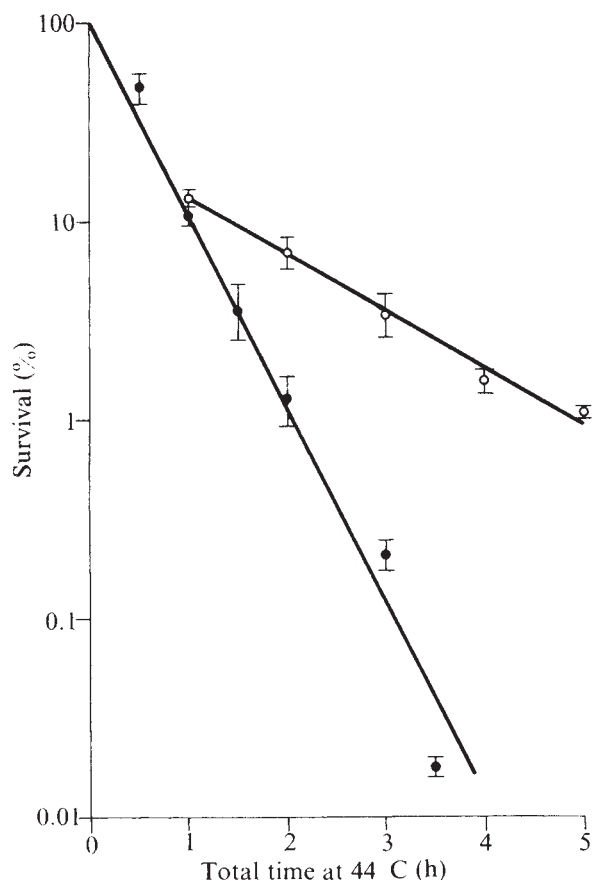


Fig. 2 Response of HeLa cells to two-dose fractionated hyperthermic treatments (each dose 44 °C for 1 h). *a*, Cellular survival when incubated at 37 °C for various times between the two (44 °C for 1 h) thermal doses. *b*, Response of cells to fractionated thermal treatments with incubation first at 0 °C and then 37 °C between doses. In these experiments, cells were treated at 44 °C for 1 h. Immediately after this treatment, the cells were transferred to 0 °C. After 1 and 2 h at 0 °C, some treatment flasks were immediately returned to 44 °C for 1 h. After 2 h at 0 °C, the other treatment flasks were transferred to 37 °C and after various times at 37 °C, were reexposed to 44 °C for 1 h. After the second 44 °C treatment, cells were collected and plated for colony formation. Control experiments showed that the immediate transition of cells from 44 °C to 0 °C did not have a lethal effect on cells. Standard errors of survival determinations are as shown.

from sublethal damage. That the increase in survival in HeLa cells (Fig. 2a) is not recovery from sublethal damage, however, is supported by two factors. First, the absence of a shoulder on the single dose-survival curve implies no capacity for sublethal damage accumulation. Second, the results shown in Fig. 1 demonstrate that when HeLa cells are challenged with 44 °C for 1 h, incubated for 2 h at 37 °C and then given graded second thermal doses, again no shoulder is evident during 4 additional hours of treatment, whereas the sensitivity of the survival response, as measured by the slope, is appreciably changed.

A comparison of the slopes of thermal survival curves with D_0 being a measure of the time for survival to be reduced to 1/e on the exponential portion of the curve at a given temperature supports this observation. Thermal treatment at 44 °C for 1 h followed by 2 h incubation at 37 °C reduces the thermal sensitivity at 44 °C of our HeLa cells by a factor of three, with the D_0 for previously unheated cells being 0.5 h and the D_0 for previously heated cells being 1.5 h. Thus, these data show that the increase in survival seen in the two-dose experiments of Fig. 2a is actually the result of a decrease in the sensitivity of cells to the second dose.

The possibility existed that the reduced sensitivity (Fig. 1) could be the result of cell progression from a sensitive cell cycle phase to a less sensitive phase, as S-phase cells are more sensitive to thermal inactivation than G₁ cells^{4,5}. Data to be published elsewhere, however, demonstrate that the thermal doses used in these survival studies inhibit cell progression of both G₁ and S-phase cells for longer periods than those described in Figs 1 and 2. This implies only minimal reassortment of cells

into different cell cycle phases during these experiments and argues against a major accumulation of cells into a resistant cell cycle phase.

The reduced thermal sensitivity of these HeLa cells, after an initial thermal dose, has two characteristics. First, the process(es) leading to the changed sensitivity does not become active at 44 °C, as the single dose-survival curve of Fig. 1 does not show a resistant tail when cells are maintained at 44 °C for 3.5 h. Second, the changed sensitivity becomes apparent only when the cells are returned to 37 °C for a time before subsequent heating. To test this latter point, additional two-dose experiments were carried out. All cells were treated at 44 °C for 1 h, after which cultures were immediately transferred to an ice bath at 0 °C for up to 2 h and then transferred to a 37 °C incubator, with the second 44 °C (1 h) thermal dose being delivered at the end of hourly intervals for up to 5 h total incubation between doses. Incubation at 0 °C (Fig. 2b), which inhibits cellular metabolic activity, inhibits the increase in survival (Fig. 2a) when cells are immediately returned to 37 °C between the two hyperthermic treatments. When cells are returned to 37 °C after 2 h at 0 °C between doses, survival increases rapidly and reaches the same survival level 2 h later as cells incubated at 37 °C immediately after the initial thermal dose.

These results suggest that the thermal resistance seen in Fig. 1 is induced by the first thermal dose, is dependent on cell metabolism and in these HeLa cells, the maximum change in sensitivity occurs by 2 h after the end of the first hyperthermic dose. Results to be published elsewhere demonstrate that the level of induced thermotolerance is dependent on both the temperature and the time at the elevated temperature of the first thermal dose and that it persists for about one cell cycle but is not passed on to subsequent generations. Figures 1 and 2 argue that our HeLa cells are not able to accumulate or recover from sublethal hyperthermic damage. Rather, a single hyperthermic treatment can induce a transient state of thermotolerance, but cannot produce heritable hyperthermic resistance.

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Regional turnover and synthesis of catecholamines in rat hypothalamus

THE occurrence of catecholaminergic systems in the hypophyseotropic area of the hypothalamus has led to numerous studies of the role of catecholamines in the control of pituitary function¹⁻⁴. The lack of biochemical methods of sufficient sensitivity to estimate catecholamine concentrations and metabolism in small brain parts has, however, hampered progress in this field. It has been claimed on the basis of histochemical fluorescence studies, that predominantly dopamine is present in the median eminence of the rat (see ref. 3). Other reports, dealing with the fluorometric determination of catecholamine concentrations, have claimed that rat median eminence dopamine levels are low relative to the noradrenaline concentrations (see ref. 3). Using sensitive radiochemical catecholamine assays, both claims were disproved^{5,6}. The considerable regional differences

in catecholamine concentrations in hypothalamic nuclei observed by Palkovits *et al.*⁶ stress that great care should be taken to properly dissect the hypothalamus, a conclusion also indicated by Kavanagh and Weisz⁷.

We present here data on regional hypothalamic noradrenaline and dopamine metabolism obtained with two methods, one involving measurement of the rate of catecholamine loss following synthesis inhibition using a sensitive radiochemical assay, and the other involving estimation of the rate of accumulation of ³H-catecholamines from L-³H-tyrosine *in vitro*.

Male Wistar rats (130-140 g) were used. The hypothalamus was dissected into median eminence, the area including the part of the arcuate nucleus dorsal to the median eminence, and the residual hypothalamus^{5,7,8}. The average weights of the dissected regions were 0.51, 1.92 and 32.6 mg, respectively.

In the first series of experiments rats were given α -methyl-p-tyrosine methyl ester HCl (α -MPT; 300 mg kg⁻¹, intraperitoneally) 4, 2 or 1 h before decapitation. Noradrenaline, dopamine and, for steady state levels only, adrenaline, were assayed in the hypothalamus parts of untreated and α -MPT-treated rats, using a radiochemical method derived from that described previously (ref. 9 and details in J.G., H.L.J.M. Wijnen and D.H.G.V., unpublished).

Table 1 summarises the concentrations of noradrenaline, dopamine and adrenaline in the three regions investigated, the rate constants of noradrenaline and dopamine loss after α -MPT and the turnover rates calculated from these¹⁰. The plots of the logarithms of catecholamine concentrations against time were linear for both amines in all three regions. The steady state levels are close to those already reported⁵⁻⁷. Although the rate constant of dopamine loss in the median eminence was somewhat lower than in the arcuate nucleus area and the residual hypothalamus, the rate of dopamine turnover in the median eminence was extremely high relative to that in the other regions. Both the rate constant of noradrenaline loss and the noradrenaline turnover rate were markedly lower in the median eminence than in the other regions. A possible explanation for this phenomenon is the occurrence of a significant amount of median eminence noradrenaline in an extraneuronal pool¹¹. The turnover of noradrenaline in the median eminence was considerably slower than that of dopamine. This is in agreement with the findings of Löfström *et al.*¹², who used fluorescence histochemistry in combination with microfluorimetry to measure catecholamine depletion in the median eminence after synthesis inhibition, and observed a much lower catecholamine turnover in the subependymal layer, supposedly containing mainly noradrenaline terminals, than in the external layer, which is presumed to contain predominantly dopamine terminals.

In the second series of experiments hypothalamus parts were incubated for 45 min in Krebs-Henseleit bicarbonate medium in the presence of L-3,5-³H-tyrosine to estimate the accumulation of tritiated catecholamines from the tritiated precursor amino acid as described previously for larger brain parts¹³.

As can be seen from Table 2, a gradient was observed in the *in vitro* accumulation of ³H-dopamine from median eminence to deeper hypothalamic structures, similar to that for the dopamine turnover rates. ³H-noradrenaline accumulation, however, was of the same order of magnitude in all three regions. This latter observation is at variance with the data obtained from the α -MPT experiments, which suggested a slower turnover of noradrenaline in the median eminence than in the arcuate nucleus area and the residual hypothalamus. This may indicate that noradrenaline synthesis in the neuronal pool in the median eminence is relatively high, and suggests one of the disadvantages of the α -MPT method, that is, that it does not allow discrimination between various amine pools in the same brain region with different turnovers. Apart from this feature the results obtained with the two methods are similar. Both methods enable discrimination to be made between noradrenaline and dopamine metabolism in very small areas of brain and can be helpful in

Table 1 Steady state level, turnover and turnover rate of noradrenaline and dopamine in rat median eminence (ME), arcuate nucleus area (ANA) and residual hypothalamus (RH)

	Tissue weight (mg)	Noradrenaline 0 h level ($\mu\text{g g}^{-1}$)	Turnover (h^{-1})	Turnover rate ($\mu\text{g g}^{-1} \text{h}^{-1}$)	Dopamine 0 h level ($\mu\text{g g}^{-1}$)	Turnover (h^{-1})	Turnover rate ($\mu\text{g g}^{-1} \text{h}^{-1}$)	Adrenaline 0 h level ($\mu\text{g g}^{-1}$)
ME	0.50 $\pm$ 0.01	3.57 $\pm$ 0.24 (6)	0.051 $\pm$ 0.006	0.185	7.64 $\pm$ 0.67 (5)	0.310 $\pm$ 0.009	2.194	NM (6)
ANA	1.93 $\pm$ 0.04	3.03 $\pm$ 0.17 (6)	0.210 $\pm$ 0.009	0.549	0.50 $\pm$ 0.06 (5)	0.470 $\pm$ 0.012	0.212	0.121 $\pm$ 0.004 (5)
RH	32.7 $\pm$ 0.4	2.54 $\pm$ 0.12 (6)	0.159 $\pm$ 0.007	0.398	0.32 $\pm$ 0.01 (5)	0.445 $\pm$ 0.009	0.118	0.046 $\pm$ 0.006 (6)

Hypothalamus parts of untreated rats and of rats which had been treated with α -MPT (H44/68, AB Biotec, Stockholm; 300 mg kg^{-1} intraperitoneally) 4, 2 or 1 h before decapitation were homogenised in 50–200 volumes 0.1 N HClO_4 . Noradrenaline, dopamine and, for steady state levels only, adrenaline were assayed in 20- μl samples of the supernatants using a radiochemical method involving the paper chromatographic separation of the ^3H -methoxy derivatives of the catecholamines obtained by incubation of the samples with a rat liver catechol-O-methyltransferase preparation and S-adenosyl-L-methyl- ^3H -methionine (Radiochemical Centre, Amersham; 5–9 Ci mmol^{-1}), (J.G., H. L. J. M. Wijnen and D.H.G.V. unpublished). Blanks, tissue blanks and internal as well as external standards were run parallel with the samples. Sensitivity of the assay for all three catecholamines was approximately 15 pg. Values have been corrected for cross interference, which amounted to less than 2%. The rate constants of amine loss (k) and turnover rates of the catecholamines were calculated according to Brodie *et al.*¹⁰. Results are expressed as mean values $\pm$ s.e.m.; number of observations for levels was as indicated in brackets; number of observations for tissue weights, k s and turnover rates was 22–24.

NM, Not measurable because of interference from high dopamine levels.

Table 2 *In vitro* accumulation of ^3H -noradrenaline and ^3H -dopamine from L- ^3H -tyrosine in rat median eminence (ME), arcuate nucleus area (ANA) and residual hypothalamus (RH)

	Tissue weight (mg)	^3H -tyrosine ($\mu\text{Ci mg}^{-1}$)	^3H -noradrenaline ^3H -tyrosine	^3H -dopamine ^3H -tyrosine
ME	0.52 $\pm$ 0.01	0.657 $\pm$ 0.029	4.37 $\pm$ 0.23	44.6 $\pm$ 3.7
ANA	1.92 $\pm$ 0.02	0.308 $\pm$ 0.014	4.89 $\pm$ 0.22	10.69 $\pm$ 0.83
RH	32.6 $\pm$ 0.3	0.035 $\pm$ 0.001	4.32 $\pm$ 0.19	6.24 $\pm$ 0.032

ME and ANA were preincubated for 10 min at 37 °C in 0.5 ml Krebs–Henseleit bicarbonate buffer, pH 7.2; RH, chopped in 0.3 mm slices with a McIlwain Tissue Chopper, in 2.0 ml of this buffer. Subsequently 50 μl buffer containing L-3,5- ^3H -tyrosine (Radiochemical Centre, Amersham) of appropriate specific activity were added and incubation was continued for 45 min. The specific activity of the ^3H -tyrosine added was 21.0 Ci mmol^{-1} (ME), 9.8 Ci mmol^{-1} (ANA) or 1.16 Ci mmol^{-1} (RH); the final concentration of tyrosine in all incubation media was 8.6 μM . The incubation was stopped by addition of 4.0 ml ice-cold buffer. After centrifugation and a wash cycle the pellets were homogenised in 0.4 N HClO_4 . Carrier tyrosine, noradrenaline and dopamine were added and ^3H -tyrosine, ^3H -noradrenaline and ^3H -dopamine were purified on Dowex 50WX4 and alumina columns as described previously¹³. Tissue medium ratios for ^3H -tyrosine were close to 3.6 in all cases. Accumulation of tritiated catecholamines from ^3H -tyrosine was linear for at least 45 min, and seemed to be directly proportional to the specific activity of the tyrosine added to the incubation medium. Thus, the ratio of ^3H -catecholamines (nCi per mg tissue) to ^3H -tyrosine (μCi per mg tissue) was taken as an index of ^3H -catecholamine synthesis. Results are expressed as mean values $\pm$ s.e.m. Number of observations was 24–27.

studies dealing with pharmacological and endocrinological manipulation and catecholamine activity in localised brain structures.

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Rate of nucleogenesis as a measure of gene activity

THE nucleolus is not a permanent organelle in the life of a cell; at the initiation of a new cycle, the nucleolar formation seems to depend on RNA synthesis directed by nucleolar RNA polymerase¹. We have compared the rate of nucleogenesis in meristem cells of *Allium cepa* L. and in those of four *Vicia* species with different numbers of ribosomal cistrons and different DNA contents^{3,4} as well as the rate of nucleogenesis in four different cell populations lying side by side in the root of *Zea mays*. The rate of nucleogenesis per ribosomal cistron seems to be constant in the different species growing in similar conditions, but this mean rate was modified in metabolically different subpopulations in the same root.

We used primary root meristems of *V. faba*, *V. narbonensis*, *V. sativa*, *V. villosa* and *A. cepa* growing at 15 °C in constantly aerated water in the dark and *Zea mays* roots growing at 23 °C in damp sphagnum, similarly in the dark (see Fig. 1). Roots (2–4 cm long) were treated for 1 h with 0.1% caffeine, which inhibits cell plate formation—cytokinesis—in those cells undergoing (during treatment) the final stages of mitosis and thus marked as binucleate at the start of a new cycle.

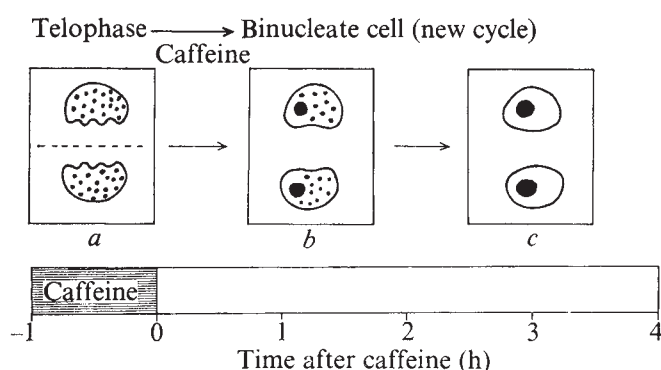


Fig. 1 Experimental scheme. Caffeine labels those cells going through telophase during treatment. Recording of the binucleate cells with fully organised nucleoli at successive hours after treatment provides a direct measure of the rate of nucleologenesis in these cells. *a*, Preenucleolar bodies in telophase; *b*, pre-nucleolar bodies and incipient nucleoli in early G_1 ; *c*, fully organised nucleoli.

The synchrony of these binucleate cells is excellent, particularly in the short term; and as nucleologenesis takes place precisely at those stages of the cell cycle which coincide with, or occur immediately after, caffeine labelling, these binucleate cells make suitable subjects to study the kinetics of nucleologenesis.

Figure 2 shows the appearance of fully organised nucleoli in the four *Vicia* species. The time at which 50% of the binucleate cells had fully organised nucleoli was estimated and the final nucleolar volumes on completion of nucleologenesis were scored (Table 1). In all species studied the maximum number of nucleoli was two, indicative of species with one pair of nucleolar organiser chromosomes. In 50% of cells these two nucleoli fused into one nucleolar mass. In *V. sativa*, however, only 3% of the nuclei showed two nucleolar masses.

We have estimated the rate of nucleologenesis per ribosomal cistron (Table 2), and found that in meristems of the four species of *Vicia* and in *A. cepa*, it is practically constant, with an average value of $2.0 \pm 0.2 \times 10^{-3} \mu\text{m}^{-3} \text{h}^{-1}$ per cistron.

The rate of nucleologenesis in stele 1 of maize roots is

Table 1 Nucleolar parameters

	Time of nucleologenesis* (h)	Nucleolar volume† (μm^3 ; mean $\pm$ s.e.)
<i>V. villosa</i>	4.7	26.3 ± 2.5
<i>V. sativa</i>	3.7	24.6 ± 2.3
<i>V. narbonensis</i>	2.9	36.0 ± 3.1
<i>V. faba</i>	3.8	79.1 ± 6.8
<i>A. cepa</i>	1.6	43.6 ± 4.0
<i>Zea mays</i>		
Cap initials	0.3	7.8 ± 0.6
Quiescent centre‡	1.1	5.4§
Stele 1	0.7	8.2 ± 0.7
Stele 2	0.5	9.8 ± 0.8

*Time when 50% of the binucleate cells have fully organised nucleoli.

†Volumes were measured the first full hour after the recorded timing of nucleologenesis (that is, in the fifth hour after caffeine in *V. villosa*; in the fourth hour in *V. sativa*, and so on).

Vicia and *Allium* roots were silver impregnated according to the scheme of Fernández-Gómez *et al.*¹⁴. This impregnation preferentially contrasts protein which accumulates in the fibrillar moieties of nucleoli and, to a lesser extent, in the granular zones¹⁵. It gives a very good nucleolar image. Squashes were prepared of the terminal 2 mm of the root, where a fairly pure meristematic zone is found. *Zea* roots were silver impregnated as above and, later dehydrated, embedded in paraffin, and sectioned at 5 μm . Median sections of 40 roots were scored. Stele 1 corresponds to steler cells just above the quiescent centre and stele 2 to a zone 200 μm further away.

‡Because of the small size of this population and its low proliferation rate, the timing of nucleologenesis has been taken from previous data, taking into account the frequency of cells with reorganising nucleoli and the cycle duration¹⁶.

§Volume corresponds to mean nucleolar volume in the zone.

similar to that shown by the meristems of *Vicia* and *Allium*, whereas the rate of nucleologenesis in a stelar zone more remote from the quiescent centre (stele 2) is higher. An intermediate value between those found in steles 1 and 2 would probably be representative of total meristematic behaviour. According to Table 2 this intermediate value would be about $2.5 \times 10^{-3} \mu\text{m}^{-3} \text{h}^{-1}$ per cistron, that is, higher than that obtained for *Vicia* and *Allium*. This may be attributable to different growing temperatures (23 °C for maize and 15 °C for *Vicia* and *Allium*), as temperature may be an important factor in determining the duration of nucleologenesis—it certainly is in determining the duration of the cycle⁵.

The fact that the nucleologenesis rate is similar in the root meristems of at least five different species could mean that all the ribosomal cistrons are operational and transcribed at a similar rate during this process. The mean rate per cistron, however, does not correspond to the optimum, as in cap initial cells in maize a rate of $4.2 \times 10^{-3} \mu\text{m}^{-3} \text{h}^{-1}$ per cistron was measured, that is, twice the average value obtained in complete meristems. Similarly, in the case of wild type *Xenopus*, 'normal' transcriptional activity is not the optimum, as studies^{12,13} of rRNA synthesis in *Xenopus* mutants deficient in ribosomal cistrons have shown that a normal transcriptional rate could be achieved with half the wild type number of cistrons.

The study of nucleologenesis in *A. cepa* in the presence of metabolic inhibitors showed that inhibition of nucleolar transcription impedes nucleologenesis. Surprisingly, protein synthesis inhibition accelerates nucleologenesis^{1,9}. Thus, nucleologenesis may be modulated by concurrent metabolic processes in the cell. In this respect, Clowes^{6,7} and Barlow⁸ have shown very different rates of incorporation of radioactive precursor into proteins and RNA between the maize populations studied. This could explain the divergence from the 'normal' rate in the quiescent centre and cap initials.

Significant differences in nucleolar transcriptional activity of ribosomal cistrons exist, for example, between pre- and postgastrula stages in *Xenopus*², and in mutants, even though this is not the case for other transcriptional modulations. Thus, Mukherjee and Beermann¹⁰ demonstrated that in *Drosophila* there was dosage compensation for the transcriptional rate in the single X chromosome in males compared with the two X chromosomes in females. Similarly Ananiev *et al.*¹¹ studied the transcriptional rate in *Drosophila* genotypes differing in the proportion of X chromosomes to autosomes and showed that the dosage compensation of enzyme synthesis coded by sex-linked genes took place during transcription.

Fig. 2 Percentage of binucleate cells with fully organised nucleoli at successive hours after caffeine treatment. Time when 50% of these cells had completed nucleologenesis may be obtained from this graph. Δ , *V. narbonensis*; $\blacktriangle$, *V. faba*; $\bullet$, *V. sativa*; $\circ$, *V. villosa*.

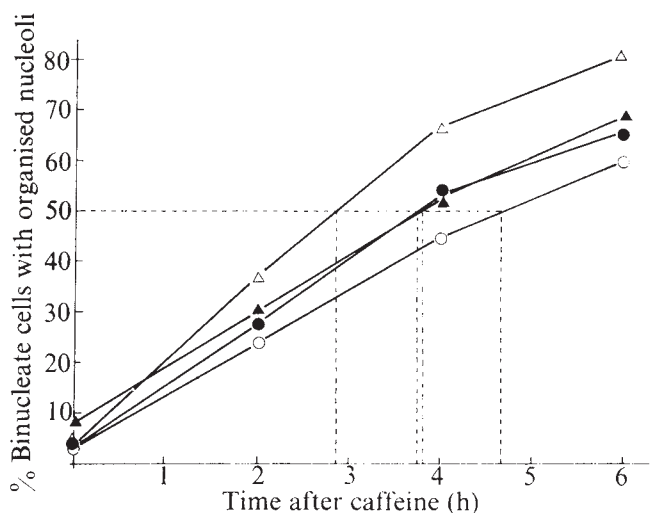


Table 2 Number of ribosomal cistrons and rate of nucleogenesis

	DNA content in 2C nuclei (pg)*	No. of ribosomal cistrons ($\times 10^{-3}$)†	Nucleogenesis rate ($\times 10^{-3}$ μm^{-3} h ⁻¹ per cistron)
<i>V. villosa</i>	3.83	2.50	2.2
<i>V. sativa</i>	3.92	3.75	1.8
<i>V. narbonensis</i>	14.38	6.25	2.0
<i>V. faba</i>	29.56	9.50	2.2
<i>A. cepa</i>	33.00	13.30‡	2.0
<i>Zea mays</i>	7.73	6.2‡	—
Cap initial	—	—	4.2
Quiescent centre	—	—	0.7
Stele 1	—	—	1.9
Stele 2	—	—	3.2

*DNA content was estimated by microdensitometry on Feulgen-stained nuclei (in a Vickers scanning microdensitometer). The value of 33 pg in 2C *A. cepa* nuclei were used for the conversion into the absolute values.

†Ref. 4.

‡Ref. 3.

We postulate that the rate of nucleogenesis may be used as a direct test of the activity of a gene. The possibility of manipulating gene action and quantifying the response modulation of ribosomal cistrons in this test remains open.

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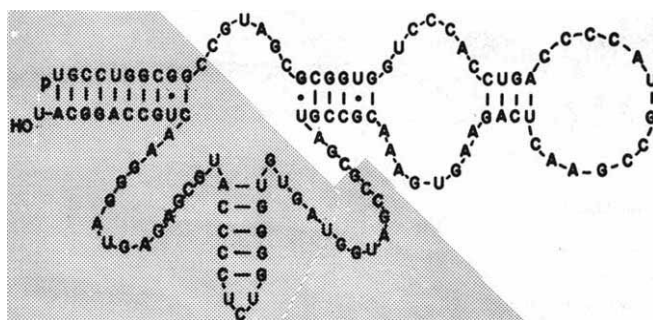


Fig. 1 Schematic representation of 5S RNA secondary structure. Normal base pairs are connected by vertical lines, whereas G–U pairs are indicated by dots. The shaded portion of the molecule is that which has been identified as being involved in an interaction with specific 50S subunit proteins^{19,20}. The structure is drawn with helices aligned along the long axis in accord with the suggestion of Connors and Beeman³⁰.

caveat. A phylogenetically broad range of prokaryotic 5S RNAs have been shown to be functionally equivalent (that is, interchangeable)^{6,7}. Thus, phylogenetic conservation of functionally significant features of the 5S RNA molecule is very likely. By including in such a study 5S RNAs isolated from organisms which occupy unusual ecological niches (extremes of temperature, pH, ionic strength, pressure or antibiotic tolerance), it should be possible not only to identify the normal constraints on the molecule's primary structure, but also to demonstrate how these constraints change under selective pressure. This in turn should provide clues to the functional significance of the constraints. A theoretical analysis of possible base-pairing schemes has been combined with an experimental programme of sequence determination to reveal the principal features of the architecture of this molecule and to provide insight into its mode of function^{8,9}.

It is the fundamental premise of this comparative approach that functionally equivalent molecules will exhibit the same secondary structure. Thus, if a helix is found in one primary sequence, it must be present in all other examples to be considered real. In addition, a base-paired region has been assumed to conform to a set of rules which are suggested by examination of tRNA secondary structures: only G–C, A–U and G–U base pairs are allowed; all helices consist of at least three base pairs without G–U pairs or four base pairs when G–U pairs are allowed; looped-out bases are assumed to be non-existent or very rare; and hairpin loops are assumed to contain at least three nucleotides.

Applying these rules, the diagonal method of Tinoco *et al.*¹⁰ is used to predict all possible helices in any 5S ribosomal RNA (rRNA) molecule. Once a given helix is found to be universal, it greatly restricts the remaining helices which might exist. In the case of *Escherichia coli* 5S rRNA, 110 possible helices exist, and all but nine of these are ruled out by the secondary structure proposed here. None of these nine, including the largest (four base pairs, 25–29 with 104–107) are, however, universal. Thus, the structure proposed here is in this sense complete.

The general prokaryotic 5S RNA (Figs 1 and 2) contains four helices, herein referred to as the molecular stalk, the prokaryotic loop, the tuned helix, and the common arm base.

The molecular stalk is in all cases a contiguous stretch of base pairs (usually 10 or 11) which serves to tie together the two ends of the molecule¹¹. The primary structure of this region is rather variable and often contains G–U pairs, for example, the three contiguous G–U pairs in the stalk of *Bacillus megaterium* 5S RNA⁸.

The prokaryotic loop, *E. coli* positions 82–86 and 90–94, has been seen only in prokaryotic 5S RNAs; it comprises four to five base pairs (*E. coli* can be extended to eight when G–U pairs are included) and generally closes a tight hairpin of three pyrimidines or four unspecified nucleotides. Although its primary structure is somewhat variable, it contains a high

5S RNA secondary structure

NUMEROUS attempts have been made to deduce a secondary structure of 5S RNA^{1–4}. Differing approaches have yielded various structures, but each turns out to be inconsistent with either physical, chemical, biochemical, or comparative evidence. Clearly the situation is not analogous to that which obtained for transfer RNA (tRNA). There is, however, at least one fundamental difference between the two molecules. The functional 5S RNA molecule is at all times an integral part of the 50S ribosomal subunit, whereas tRNA is a transient inhabitant of the ribosome milieu. Thus, it is possible that functional conformations of the 5S RNA molecule need only exist in the context of the ribosome, and consequently, may only exist therein. Thus, the interpretation of experimental investigations on the isolated 5S RNA molecule may have inherent difficulties.

A comparative analysis⁵ of 5S RNA primary structure is one approach to defining functionally significant secondary (and tertiary) structure whose interpretation is not open to the above

		10		20	30		40		50	
<i>E. coli</i>	p	UGCCUGGCGG	CCGUAGC	GCGGUG	GUCC [CAC	CUGA	CCCCAUGCCGAAC	UCAG	AAGUGAAA]	
<i>Photo. 8265</i>	p	UGCUUGGCGA	CCAUAGC	GUUAUG	GACC [CAC	CUGA	UCCCUUGCCGAAC	UCAG	UAGUGAAA]	
<i>Pseudo. fluor.</i>	p	UGUUCUUUG	ACGAGUAGUA	GCAUUG	GAA [CAC	CUGA	UCCCAUCCCGAAC	UCAG	AGGUGAAA]	
<i>B. megaterium</i>	p	UCUGGUGGCG	AUAGC	GAAGAG	GUCA [CAC	CCGU	UCCCAUACCGAAC	ACGG	AAGUUAAG]	
<i>B. stearrowthermophilus</i>	p	CCUAGUGACAA	UAGCGA	G·AGAG	GAAA [CAC	CCGU	UCCCAUCCCGAAC	ACGG	AAGUUAAG]	
<i>A. nidulans</i>	pU	CCUGGUGUC	UAUGGC	GGUAUG	GAAC [CACU	CUGA	CCCCAUGCCGAAC	UCAG	UUGUGAAA]	
<i>Chlorella</i>	ppp	AUGCUACGUU	CAUA·C	ACCACG	AAAG [CAC	CCGA	UCCCAU CAGAAC	UCGG	AAGUAAAA]	
KB cell	ppp	GUCUACGGC	CAUACC	ACCCUG	AACG [CGC	CCGA	UCUCGU CUGAUC	UCGG	AAGCUAAG]	
<i>X. laevis</i>	ppp	GCCUACGGC	CACACC	ACCCUG	AAAG [UGC	CCGA	UCUCGU CUGAUC	UCGG	AAGCCAAG]	
<i>T. utilis</i>	pp	GGUUGCGGC	CAUAUC	UAGCAG	AAAG [CAC	C·GUU	CUCCGU CCGAUC	AACUG	UAGUUAAG]	
		A		B		C			C'	
		60	70	80		90	100		110	120
<i>E. coli</i>		CGCCGU	AG [CGCCGAUGGUAGU]	G UGGGG	UCU	CCCCA	U GCGAGAGUAGGGAA		CUGCCAGGCA	U _{OH}
<i>Photo. 8265</i>		CGUAAU	AG [CGCCGAUGGUAGU]	G UGGGG	UCU	CCCCA	U GUGAGAGUAGGACA		UCGCCAGGCA	U _{OH}
<i>Pseudo. fluor.</i>		CGAUGC	AU [CGCCGAUGGUAGU]	G UGGGG	UUU	CCCCA	U GUCAAGAUCUCGAC		CAUAGAGCA	U _{OH}
<i>B. megaterium</i>		CUCUUU	AG [CGCCAAUGGUAGU]	U GGGAC	UUU	GUCCC	U GUGAGAGUAGGA		CGUUGCCAGG	C _{OH}
<i>B. stearrowthermophilus</i>		CUCUCC	CAG [CGCCGAUGGUAGU]	U GGGGC	CAGC	GCCCC	U GCAAGAGUAGG		UUGUCGCUAGG	C _{OH}
<i>A. nidulans</i>		CAUACC	UG CGGCAACGAUAGC	UC CCGG	GUAG	CCGG	UCGCUAAAAUAGCUC		GACGCCAGG	UC _{OH}
				D		D'				
<i>Chlorella</i>		CGUGGU	UGGG CUCGA	CUAGUACU (GGGUU)GGAGGAUUACCUGAGUGGG (AACCC)C					GACGUAGUGU	OH
KB cell		CAGGGU	CGGG (CCUGGU)	UAGUACU UGGAU GGGAGACCGCCUGGGAAU (ACCGGG)U					GCUGUAGGC	U(U)U _{OH}
<i>X. laevis</i>		CAGGGU	CGGG (CCUGGU)	UAGUACU UGGAU GGGAGACCGCCUGGGAAU (ACCGGG)U					GUCGUAGGC	UUU _{OH}
<i>T. utilis</i>		CUGCUA	AGAG (CCUGA)UCGAGUAGU	GUAGU GGGUGACCAUACGCGAAAC (UCAGG)U					GCUGCAAUC	U _{OH}
		B'							A'	

Fig. 2 Ten known sequences of the 5S RNA molecule which differ substantially are divided into two groups—corresponding to prokaryotes and eukaryotes^{1,8,9,11,17,18,31-35}. The alignment is adjusted to juxtapose secondary structural features. Vertical lines indicate regions of base pairing and brackets enclose regions of high phylogenetic conservation. Parentheses indicate regions of base pairing in the 3' half of the eukaryotic molecule^{1,17}. The sequences are as published elsewhere with the exception of *B. stearrowthermophilus*, the unsequenced portions of which are arranged in a manner consistent with the proposed structure. In this case also, a correction is included—the oligomer UCUCUACCCG is replaced by UUCUCAUCCCG. Position numbers in the *E. coli* sequence are included as subscripts, as used in the text.

portion of G-C pairs in those cases so far examined; and in the case of *E. coli*, has been reported to be rather resistant to nuclease attack¹¹. This loop, too, has been included in many previous models, although a possible alternative pairing between *E. coli* positions 33-42 and 79-88 has frequently been suggested¹². Comparative study makes the latter unlikely (as both the precise location and length of the pairing would have to

vary considerably). Until this alternative can be eliminated, however, the proposed structure cannot be claimed to be unique.

The third helix, *E. coli* positions 18-23 and 60-65, is referred to as 'tuned' because its thermodynamic stability as estimated by the method of Tinoco *et al.*¹³ seems to be under energetic constraints. This constraint is quite unique to this helix and varies from extreme weakness in marine prokaryotes (four

examined so far), to intermediate values in non-marine prokaryotes, and to quite stable structure in all eukaryotes⁹. As the total length of this helix, six base pairs, is highly conserved (*B. stearothermophilus* represents either an exception or a sequencing problem), the energetic control is obtained by significant variations in composition. The terminal G-C pair (*E. coli* positions 23 and 60) is, however, found in all organisms examined so far.

Finally, the common arm base, *E. coli* positions 31–34 and 48–51, is so termed because it closes a 12–13 base loop which contains in all prokaryotes examined so far the sequence CGAAC, which reportedly interacts with the tRNA common arm sequence GTΨCG (refs 14–16). The common arm base comprises four base pairs in all cases except yeast, which seems to contain only three^{17,18}. If the loop closed by this helix does interact with the common arm of tRNA, a contiguous stretch of at least eight base pairs could result (four within the 5S RNA and four or five between the tRNA and 5S RNA). The effect of the formation of such an intermolecular helix on the tRNA common arm geometry and thus overall tRNA tertiary structure would be quite drastic.

Whereas primary structure is not generally highly conserved in the four double-stranded regions, two single-stranded regions in the molecule are, in fact, strongly conserved. The smaller of these, GCGCC^aAUGGUAGU (*E. coli* positions 67–80), is found in that region of the molecule protected by three 50S ribosomal proteins (see Fig. 1)^{19,20}. This sequence is found in all published bacterial 5S RNA structures, but is not entirely universal, being absent in the eukaryotes and *Anacystis*. The second, larger region of high conservation, positions 28–59 in *E. coli*, includes the common arm base and, near its middle, the universal prokaryotic sequence CGAAC. The molecule (Fig. 1) can thus be crudely pictured as consisting of two regions, both containing strongly conserved sequences; one of recognised functional importance (5S–tRNA interaction), the other apparently of structural importance (perhaps 5S interaction with the 50S particle). Separating these two large regions is the tuned helix.

Two additional general constraints which have been observed in the nine existing 5S RNA sequences are: the first four nucleotides in the loop defined by the common arm base, although variable, are in all cases pyrimidines (four different examples thus far); and a stretch of at least four purines exists about twenty nucleotides from the 3' end. Pace *et al.* have suggested the latter to be a recognition signal for a posttranscriptional modification enzyme in *B. subtilis*^{21,22}.

The proposed model for prokaryotic 5S RNA secondary structure is quite consistent with the comparative evidence. Each helix has been substantiated by examples wherein significant base changes in its primary structure have occurred without violating the proposed base-pairing constraint. In addition, it is in agreement with most of the evidence obtained by physical and chemical methods. In particular, the recent high resolution nuclear magnetic resonance prediction of 28 base pairs, including four A–U in *E. coli*, is in excellent agreement with the proposed structure, that is, 25 base pairs, five of which are A–U. Chemical modification studies using a variety of reagents yield no examples of definitively placed modified nucleotide residues that occur in proposed double-stranded regions.

Oligonucleotide binding studies²³, however, suggest that in *E. coli* regions 9–10 and 59–65—both involving proposed helices—can pair to external tri- and tetranucleotides. The interpretation that these regions are, therefore, not base paired in the intact molecule is based on the assumption that external tri- and tetranucleotides cannot perturb secondary structure. This assumption seems questionable in the former instance at least where the final two base pairs of the molecular stalk (one a G–U pair) would be replaced by a tetramer pairing that not only stacks on the remaining (eight) pairs of the stalk, but consists itself of four G–C pairs. Also, if Lewis and Doty²⁴ were not working with the native or A form of 5S RNA, their

conclusion regarding region 59–65 would not apply to that form.

The results of Vigne and Jordan²⁵ are also inconsistent with the present model only if one assumes that two or more breaks in the molecular backbone never disrupt secondary structure, which again is highly questionable.

Although the eukaryotic 5S RNA molecule is quite similar¹, it is distinctly different from its prokaryotic counterpart in that it lacks the prokaryotic loop and the universal sequence CGAAC. This is highly suggestive of a subtle divergence in the mechanism of protein synthesis which has been consistently hinted at in other comparisons of eukaryotic and prokaryotic ribosomes²⁶.

We feel that the tuned helix has a crucial role in 5S RNA structure and, consequently, function. Perhaps the most convincing evidence of this comes from studies involving the 'native' (and closely related A form) against the B form of this molecule^{24,27}. There are specific points of chemical modification characteristic of each form²⁸. Similarly, defined points readily accessible to enzymatic attack characterise each form²⁹. One notable difference between the two forms is the accessibility to attack of bases in the tuned helix; that is, in the native form they seem to be paired, whereas they seem to be uncoiled in the B form. Although there is at present no evidence that these two forms are functionally significant, it does seem clear that the tuned helix is thermodynamically sensitive and is a major determinant in 5S RNA conformation.

This observation, the apparent energetic constraint, and the central position of the tuned helix suggest that the coiling and uncoiling of that structure is a key element in 5S RNA function. As the tuned helix could control the position of the common arm base which it encloses, it could therefore control the position and/or conformation of the tRNA molecule. Thus, this coiling/uncoiling event would make possible a cyclic (and possibly time-symmetric) interaction of the 5S RNA with the two tRNA molecules during protein synthesis.

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Differential effect of plasma fractions from normal and tumour-bearing rats on nuclear RNA restriction

WE have already presented preliminary evidence¹ that crude plasma from tumour-bearing rats contains elevated concentrations of macromolecules which stimulate the release of messenger RNA (mRNA) from isolated nuclei. The detection of these putative regulatory components was facilitated by a cell-free system developed originally to study messenger²⁻⁵ and ribosomal^{6,7} RNA (rRNA) processing and transport *in vitro*. Since both maximal RNA transport and normal nuclear RNA restriction *in vitro* (that is, equivalence between messengers released from nuclei *in vivo* and *in vitro*) require the presence of macromolecules from homologous cytosol^{2,8} it is possible that the tumour cell cytoplasm is the source of the putative regulatory macromolecules in the peripheral blood of tumour-bearing animals. This possibility is supported by evidence^{9,10} that factors involved in the regulation of cellular proliferation are released to circulation from tumour cells, and by the observation¹¹ that the enzyme composition of the host liver of tumour-bearing rats converges to that of the tumour. The spectrum of messengers transported from normal rat liver nuclei in homologous cytosol is significantly different from that transported in hepatoma cytosol³, suggesting that differences exist in these putative regulatory components of the two tissues.

This study evaluates the relative RNA transport activity of the plasma from normal and tumour-bearing rats following partial purification of the putative regulatory components on a DEAE-cellulose column and attempts to rationalise their increase in tumour-bearing animals. Sixfold higher transport activity of a plasma fraction from tumour-bearing rats is relevant to the problem of the host-tumour relationship and emphasises the potential usefulness of this assay in diagnosis. The differences in the RNA sequences transported in response to plasma fractions from normal and tumour-bearing rats is compatible with the theory that some originate from the tumour cells.

Heparinised plasma from normal rats of the Sprague-Dawley strain, or from rats bearing the Novikoff ascites hepatoma intraperitoneally, or the Morris hepatomas 9618A or 5123D intramuscularly in the hind legs¹², was passed through a Sephadex G-25 column before testing or fractionation on DEAE-cellulose. The cell-free test system contained normal rat liver nuclei (prelabelled *in vivo* for 30 min with 6-¹⁴C-uracil) in homologous cytosol fortified with components (see Fig. 1). The concentration of the components were identical to the standard nuclear restriction assay^{3,4} with the exception of cytosol protein, which was reduced from a concentration of 12.0 to 4.0 mg ml⁻¹; this modification increased the sensitivity of the assay to exogenous sources of putative regulatory components, yet was sufficient to maintain both an effective concentration of ribonuclease inhibitor and the integrity of the nuclei⁷. The labelled RNA transported in 30 min at 30 °C, the bulk of which is messenger²⁻⁵, was precipitated from the nuclei-free supernatants in the presence of carrier yeast RNA with 5% trichloroacetic acid, washed with 95% ethanol and assayed in liquid scintillant. The sequence homology of the transported messengers was determined by competitive RNA-DNA hybridisation as described previously^{3,4,8}. Shown in Fig. 1a is the DEAE-cellulose elution profile of protein (A_{280}) in plasma from normal rats (controls) and rats bearing the Morris hepatoma 5123D (approximately 1.0 cm in diameter; bilateral), which has a transplant generation time of 1 month. The plasma elution profiles (not shown) of rats bearing the Morris hepatoma 9618A (approximately 1.0 cm diameter; bilateral) or the rapidly growing Novikoff

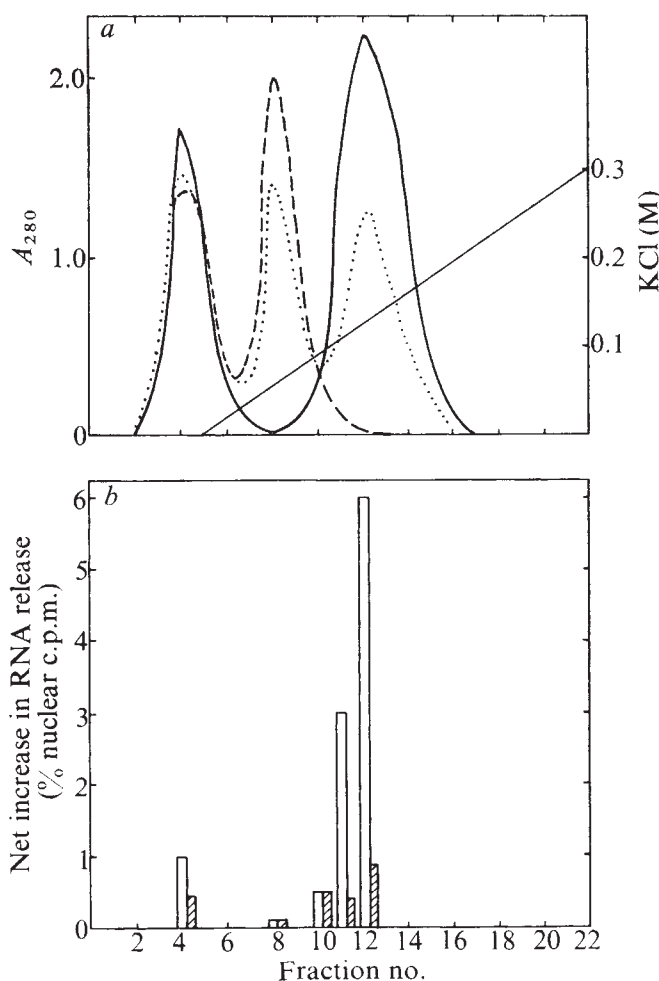


Fig. 1 *a*, DEAE-cellulose elution (A_{280}) profiles of plasma proteins from normal rats (---), bearing an 18-d (non-palpable) hepatoma 5123D (....) and rats bearing a large (1.0 cm) hepatoma 5123D (—). In each case, the equivalent of 1.0 ml of plasma was passed through a Sephadex G-25 column and the proteins were adsorbed on a DEAE-cellulose column (1.0 × 20 cm) equilibrated with 0.02 M K_2HPO_4 (pH 7.4); the adsorbed protein was eluted with a linear (0.0–0.5 M) gradient of KCl in 0.02 M phosphate buffer and 3.0 ml fractions were collected. *b*, Enhanced mRNA release in response to the plasma fractions in *a*. The data shown in the bar graph represent the net enhancement of mRNA release from prelabelled nuclei as % nuclear c.p.m. by the addition of the minimal transport system of 0.3-ml aliquots of fractions 4, 8, 10, 11, or 12 of plasma from normal (hatched bars) or tumour-bearing (open bars) rats. The minimal transport system contained 4.5×10^6 prelabelled liver nuclei per ml of medium containing 4 mg ml⁻¹ of dialysed cytosol protein, 50 mM Tris HCl (pH 7.6), 25 mM KCl, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, 0.3 mM $MnCl_2$, 5.0 mM NaCl, 2.5 mM K_2HPO_4 , 5.0 mM spermidine, 2.0 mM dithiothreitol, 3.0 mM ATP, 2.5 mM phosphoenol pyruvate, 35 units of pyruvate kinase and 300 mg of yeast RNA per ml, the total volume of the assay being 5.0 ml. The incubation was carried out at 30 °C for 30 min with the further addition of 1.0 mM phosphoenol pyruvate after 10 min and again after 20 min of incubation. To determine the percentage total of labelled nuclear RNA released during incubation, the RNA was precipitated from the nuclei-free supernatants in the presence of carrier yeast RNA with 0.1 volume of 50% trichloroacetic acid, dissolved in solubiliser and counted in liquid scintillant⁸. During a 30-min incubation the release of total nuclear counts ($\pm$ s.d.) for the minimal system was 4.7 ± 0.2 . Results are tabulated in terms of increment in % nuclear c.p.m. transported in the presence of the added plasma components over that released to the minimal system, and represent the average of two determinations which duplicated to within 10%. Essentially similar results were obtained whether or not the whole plasma was purified of low molecular weight components on Sephadex G-25. The experiments on normal serum and on hepatoma 5123D-bearing rats duplicated to within 10%. They were also confirmed using hepatoma 9618A and the Novikoff hepatoma-bearing rats.

hepatoma, with transplant generation times of 8 months and 1 week, respectively, were identical to that of the 5123D hepatoma. Also shown is the protein elution profile of the plasma from hepatoma 5123D, 18 d after inoculation, that is, when the tumour is not yet palpable. The protein elution profiles (Fig. 1) of the plasma from normal and hepatoma-bearing rats are markedly different. Thus, whereas the peak elution in the void volume (fractions 2–6) is common to the plasma of both the normal and tumour-bearing rats, the other two peaks are not. In fact, the protein components eluting at a mean KCl concentration of 0.05 M (fractions 7–10) disappears during the growth of the tumour, being decreased in the plasma from animals during the early stages of tumour growth and essentially absent during the later stages. In contrast, the protein components eluting at a mean KCl concentration of 0.125 M may be present at a very low level or essentially absent in normal rat plasma, but increase markedly during the development of the tumour. Identical results were obtained with animals bearing the Novikoff hepatoma, or hepatoma 9618A.

Shown in Fig. 1b is the ability of selected DEAE-cellulose fractions of plasma from a normal rat and a rat bearing hepatoma 5123D (tumour diameter of 1.0 cm) to enhance the transport of mRNA in the cell-free system. Fractions 4, 8 and 10 from both the normal and tumour-bearing animals showed comparable, but low transport activity. In contrast, fractions 11 and 12 had marked RNA transport activity when the plasma was derived from the tumour-bearing rat; the activity of the corresponding fractions from normal plasma was approximately one sixth that of plasma from the tumour-bearing rat. Again, the results obtained with rats bearing the Novikoff and 9618A hepatomas were identical to those reported for hepatoma 5123D. In contrast to the sixfold difference in transport activity observed when fractions 11 or 12 of the plasma from normal and tumour-bearing rats were compared, a difference of only threefold was observed when the crude plasmas from the two sources were compared.

The relationship, if any, between the disappearance during tumour growth, of the protein peak eluting from DEAE-cellulose at 0.05 M KCl and the appearance of the protein peak eluting at 0.125 M KCl, as well as the mechanisms underlying these changes are at present unknown. The possibility that the components eluting at 0.05 M KCl modify the transport activity of components eluting at 0.125 M KCl, however, was tested in the concentration range 1.0–3.0 mg ml⁻¹, with negative results.

The differential effect of active plasma fractions from normal and tumour-bearing rats on nuclear RNA restriction *in vitro* was tested by competitive RNA-DNA hybridisation. ¹⁴C-labelled RNA transported from prelabelled liver nuclei in response to 4.0 mg ml⁻¹ of homologous cytosol protein, fortified with 1.0 mg ml⁻¹ of protein from fraction 12 (Fig. 1a) obtained by chromatographing the plasma of a Novikoff hepatoma-bearing rat, was competed against unlabelled RNA transported from unlabelled liver nuclei in response to homologous cytosol protein (4.0 mg ml⁻¹) plus fraction 12 (1.0 mg ml⁻¹), derived from normal plasma. Only 75% of the messenger-like RNA sequences transported were identical in both systems, 25% of the messenger-like RNA transported in the presence of the plasma protein fraction from normal animals being different from that transported in the presence of the comparable fraction from tumour-bearing rats. Furthermore, analysis of the transported labelled RNA in sucrose density gradients (results not shown) indicated that mRNA transported in the presence of plasma fractions of the hepatoma-bearing, in contrast to the normal rats, had a size distribution which was distinctly skewed to the heavier species; this abnormal size distribution was not normalised by active fractions from normal plasma.

Both the hybridisation and density gradient studies suggest that there are qualitative differences in the regulatory macromolecules of the plasma from normal and tumour-bearing rats; it is not merely an increase in normal components. Since

analogous differences were observed³ between the RNA transported to normal and tumour cytosols, some of the plasma components may originate from the tumour. It has been established^{13,14} that certain RNA sequences are confined to the nucleus in normal cells but that this restriction is lost in cancer cells¹⁵. It has also been shown¹¹ that the enzyme composition of the host liver in animals bearing tumours shows a convergence to that of the corresponding tumour. Thus, our finding in the circulation of a regulatory component altering nuclear restriction is not surprising. Furthermore, there are several^{16,17} recent reports of specific serum components disappearing as a tumour develops although as yet neither these components nor the factor eluting from DEAE-cellulose at 0.05 M KCl have been well characterised.

Although normal plasma also contains components which enhance RNA transport, they are present in low concentration compared with tumour plasma and elicit the transport of messenger-like RNA species which are different from those transported in the presence of plasma from tumour-bearing rats. Furthermore, the protein peak containing the highest transport activity in the plasma from tumour-bearing rats is essentially missing from normal plasma; it is also low (non-detectable) in the plasma of rats with regenerating liver (unpublished observations). These results strongly suggest, but do not prove that the increased transport activity in plasma from tumour-bearing animals is due to the release of macromolecules from tumour cells to circulation. The observation that the difference in transport ability of plasma from normal and tumour-bearing animals is enhanced by preliminary fractionation on DEAE-cellulose is relevant to the adaptation of the nuclear RNA restriction assay as a tumour detection test. But further correlative studies on animals bearing a wide range of transplantable, induced primary and autochthonous tumours of various sizes are required to establish the usefulness and limitations of this test system.

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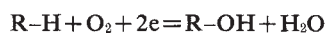
Early role during chemical evolution for cytochrome P450 in oxygen detoxification

It is generally held that early in the evolutionary history of the Earth, when abiogenic chemicals were forming associations possessing attributes by which we now define living organisms, this planet had a reducing atmosphere. The

subsequent appearance of free or molecular oxygen must have had a profound effect on such poorly specialised life forms and many would have perished. It was long assumed that the adaptive enzymatic changes of the successful forms were chiefly the elaboration of the respiratory chain enzymes, peroxidases and catalase. Research on superoxide dismutase¹ has suggested that this enzyme may also have controlled oxygen toxicity in ancient living tissue. We have studied cytochrome P450, which also metabolises oxygen, and believe that this protein may possibly be more primitive than those considered previously and may even have emerged before the advent of atmospheric oxygen.

Cytochrome P450 is an unusual haemprotein found in animals, plants and microorganisms. In animals, this *b*-type cytochrome is membrane-bound and is present in the mitochondria of certain endocrine glands and in the microsomes of cells of, among others, certain endocrine glands and the liver. Microsomal cytochrome P450 is the major hepatic haemprotein, reaching concentrations of 0.1 mM in the cell or five to ten times that of any mitochondrial (respiratory chain) cytochromes². In certain conditions, cytochrome P450 constitutes 20% of the microsomal protein or 2–3% of the hepatic protein content². The *Pseudomonas* enzyme, in contrast, is a soluble protein^{3,4}.

The cytochromes P450 possess active sites effecting hydroxylation reactions



The two electrons or reducing equivalents are furnished by NADPH and (or) NADH (ref. 5). The first reduction is of a flavoprotein but iron-sulphur proteins also participate in electron transfer for mitochondrial and bacterial hydroxylases^{5,6}. The biological functions of cytochrome P450-dependent hydroxylases include the hydroxylations (often detoxifications) of many structurally dissimilar substances such as steroids and fatty acids and xenobiotics such as drugs, carcinogens, pesticides, cannabinoids and a variety of substituted and unsubstituted hydrocarbons^{5,7–10}. Cytochrome P450 may also act as a microsomal peroxidase¹¹.

Both iron-sulphur proteins and cytochrome P450 possess low redox potentials^{6,12,13}, close to that of the hydrogen electrode (–420 mV), unlike other cytochromes and other terminal oxidases. They could thus have functioned enzymatically at a time when the Earth's atmosphere was mainly composed of hydrogen, nitrogen, methane, ammonia, carbon monoxide and carbon dioxide^{14,15}, and also after free oxygen had appeared. Both are relatively small, single polypeptides (iron-sulphur proteins, molecular weight 6,000–12,000; bacterial cytochrome P450, 45,000) with low isoelectric points; have a large content of amino acids which can be synthesised abiologically¹⁴, and can be reconstituted from their apoproteins *in vitro*^{4,16,17}. Both types are widely distributed in microorganisms, plants and animals^{5,6,18} and participate in the metabolism of a large range of substrates^{5,6,9}. The above characteristics have led some to consider that the iron-sulphur proteins may have been among the earliest to evolve and that this process took place in reducing conditions⁶.

The gradual appearance of molecular oxygen would have led to the development in primitive organisms of biochemical mechanisms either as lines of defence (for example, utilisation of porphyrins¹⁹) or to benefit from the alteration in environment (for example, development of the energy-yielding respiratory process). Although molecular oxygen is itself damaging to living matter (for example, enzymes which are labile to aerobic conditions¹⁷, autoxidation of lipids and oxidation of free sulphhydryl groups of proteins), products of oxygen metabolism such as superoxide anion, H₂O₂ and OH[•] are also highly reactive and toxic to the living fabric. It seems unlikely that the toxicity of

oxygen^{19,20} is caused by a single toxic action, and the development of tolerance is probably multifactorial²⁰. The rate of production of the superoxide radical by some enzymes has also been shown to depend on oxygen tension²⁰. The early organism may thus have developed different lines of defence against damage by the steadily increasing atmospheric oxygen. Initially, cytochrome P450 alone may have been adequate to 'mop up' traces of unwanted oxygen which entered the cell. Later, cytochrome P450 activity may not have been adequate by itself to remove all the excess oxygen but would have still performed a valuable 'buffering' or attenuating role in lowering the production of H₂O₂ and superoxide anion to within limits which could be effectively detoxified by other enzymes. This activity may also have provided the time necessary for the induction of enzymes such as superoxide dismutase¹ whenever the organism was challenged by elevated oxygen levels in the immediate environment. Occasionally, enzyme systems may produce under certain chemical constraints some reactive metabolites of oxygen such as H₂O₂ and superoxide anion. These would have been balanced by the concerted action of enzymes such as cytochrome P450, superoxide dismutase and catalase, thus reducing the longevity of these toxic species in the cellular substance. Catalase is possibly of lesser importance in protecting bacteria against oxygen toxicity¹ and the innocuous nature of the products of the activity of cytochrome P450 may be of advantage. The superoxide anion and peroxide which may be produced in certain conditions *in vivo* by cytochrome P450-dependent enzyme systems (but see ref. 11), however, would at that time, as now, have required rapid detoxification by superoxide dismutase, peroxidase and catalase.

The apoprotein of cytochrome P450 could have been derived from abiogenic amino acids, and Calvin¹⁶ has discussed how certain haemproteins may have been formed abiologically. The system would have been insensitive^{7,8} to the levels of cyanide present in the primeval oceans^{14,15}. Chemical (or photochemical^{6?}) reduction of an associated iron-sulphur protein suffices for the supply of electrons to cytochrome P450 in the absence of flavoproteins and reduced cofactors⁶. Although such reduction of an associated protein may have produced electrons for early cytochrome P450-dependent activities, Oparin²¹ considers that cofactors must have appeared very early in chemical evolution. It is also relevant that the haemprotein complexes with phospholipids to form a proteolipid which is soluble in hydrocarbon solvent and may be functional in lipid surroundings²². This could be pertinent to the theories that primordial cells were highly lipid in nature¹⁵.

The carbon monoxide in the atmosphere and the absence of oxygen^{14,15} would have made unlikely the activity of cytochrome P450 as a hydroxylase very early in the evolution of life although the photochemical action spectrum of these hydroxylation reactions⁵ has shown that illumination at certain wavelengths does reverse the inhibition caused by carbon monoxide. The solar energy which traversed the primitive atmosphere to reach the Earth's surface was very large^{14,15}—sufficient to dissociate any carbon monoxide bound on to early forms of cytochrome P450. Cytochromes are proposed to have been among the active proteins which had evolved before the advent of the oxygen atmosphere^{23,24}, and, in spite of the aerobic conditions in the liver cell, a subsidiary function of the contemporary hepatic microsomal enzyme may be in reductive reactions¹⁰. The low redox potentials of this cytochrome and associated proteins would have enabled similar reductive activities of early cytochrome P450 to have taken place in an anaerobic hydrogen atmosphere. Contemporary cytochrome P450 may also have a role in nitrogen reduction and appears in *Rhizobium* sp. bacteroids by the time of active nitrogen fixation, which requires both nitrogen and oxygen^{5,25}. Although research on cytochrome P450 has focused mainly

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The figure consists of two panels, (a) and (b), each showing an autoradiograph of a gel after electrophoresis. In both panels, bands are represented by shaded or outlined shapes, while unexposed areas are white.

- Panel (a):** Shows several distinct bands. On the left side, from top to bottom, they are labeled: 90-92, 140-141, 47-56, 32-40, 78-89, 12-16, and 57-61. On the right side, from top to bottom, they are labeled: 108-114, 115-127, 62-77, and X-Y. A vertical arrow on the far right points upwards and is labeled "Chromatography". Below the gel, a horizontal arrow points to the right and is labeled "Electrophoresis". There are symbols * and + at the bottom right.
- Panel (b):** Shows a more complex pattern of bands. On the left side, from top to bottom, they are labeled: (γ 105-110)-, (γ 105-107)-, (γ 103-110)-, (γ 104-110)-, (γ 115-118)-, 89-89, (γ 115-121)-, (γ 115-121)-, (γ 128-132), and (γ 131-132). On the right side, from top to bottom, they are labeled: (γ 105-110)-, 51-52, 133-136, (γ 112-114), (γ 108-110), (γ 87-89), (γ 86-88), 131-132, (γ 122-127), 93-99, -74-77, (γ 90-91), (γ 89-91), and 64-65. A vertical arrow on the far right points upwards and is labeled "Chromatography". Below the gel, a horizontal arrow points to the right and is labeled "Electrophoresis". There are symbols * and + at the bottom right.

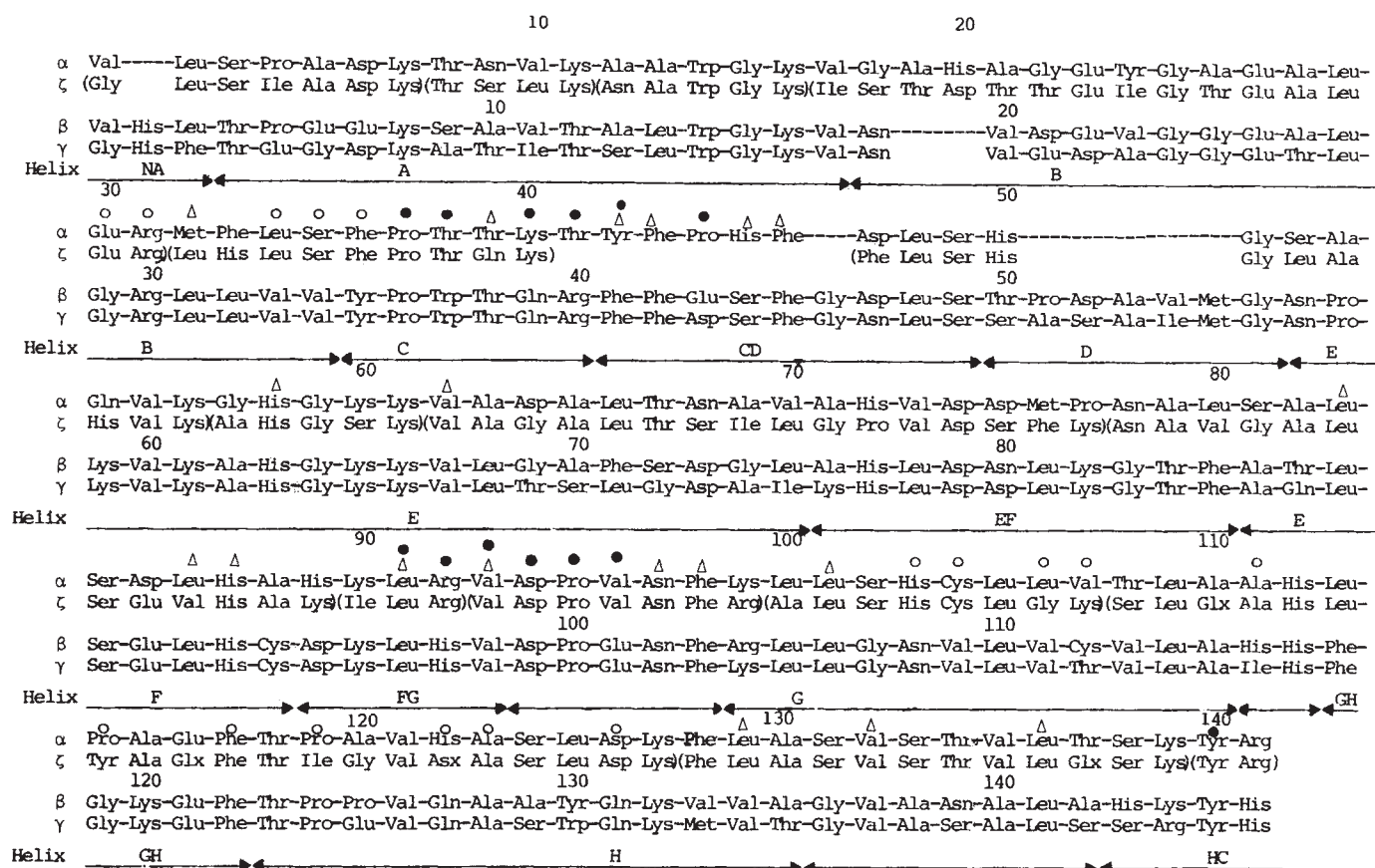


Fig. 2 Alignment of ζ -chain peptides with the amino acid sequences of three human haemoglobin chains. The chains have been arranged so as to maximise homologies, also taking into account the polarity and size of the residues, the haem contacts and the intramolecular contacts of α and β chains according to the model of Perutz as applied to man^{19,20}. The alignment suggests that the ζ chain is very closely related to the α chain. ○, $\alpha 1\beta 1$ contacts; ●, $\alpha 1\beta 2$ contacts; Δ, haem contacts.

oxygen dissociation curves, Huehns and Farooqui⁸ concluded that the ζ chain ought to resemble the α chain.

We have examined human embryos aged 5–10 weeks and neonates with the Hb Bart's hydrops foetalis syndrome for Hb Gower 1 and Portland. The Hb Gower 1 fraction contains α chains and therefore Hb Gower 1 is unlikely to be a tetramer of ϵ chains. In the case of Hb Portland we have been able to obtain further evidence for resemblance of the ζ chain to the α -chain.

We obtained blood from 67 embryos. The red cells were washed several times in excess physiological saline and haemolysed as previously described⁹. The haemolysates were pooled and chromatographed on DEAE-Sephadex¹⁰ and the fractions eluting earlier than Hb A₂, were pooled, concentrated by ultrafiltration, then electrophoresed on paper at pH 8.9 to isolate the embryonic haemoglobins. Fractions corresponding to Hb Gower 1 and Gower 2 were eluted with water and pooled separately and concentrated by ultrafiltration at 40 °C.

When the Gower 1 fraction was chain separated on a CM cellulose column¹¹ the major peak which was obtained gave on subsequent fingerprinting and amino acid analysis, peptides with amino acid compositions consistent with the structure of carbonic anhydrase B¹². A tryptic dipeptide Gly-Arg which has been cited as an example of an ϵ -chain peptide¹³, is also found in carbonic anhydrase B. The peak in position of the α chain was a haemoglobin α chain from fingerprinting and amino acid analysis of all soluble peptides.

To investigate the ζ chain, blood was obtained from two Hb Bart's hydrops foetalis neonates and Hb Portland was isolated by paper electrophoresis; haem was then removed and the globin dried under nitrogen⁹; 8 mg were digested with trypsin

and pepsin and fingerprints prepared (Fig. 1); 1 mg was used for the terminal amino acid sequence, using the modified dansyl technique of Gray^{14,15}. Glycine only was released. Most of the peptides reported by Capp *et al.*⁵ were obtained and the alignment of peptides is similar to that reported previously⁶. We have been able to align 14 additional residues and the only part of the α chain for which no peptide suitable for alignment was found are residues 41–46. We have, however, interchanged Phe and Leu for positions 47 and 52, because of the alignment of a dipeptide Gly-Leu to positions 51–52 (Fig. 1).

It is suggested from the electrophoretic mobility of peptide X aligned with residues 1–7, that the N terminus may be blocked, possibly through acetylation as it is in some γ chains. The corresponding non-blocked peptide with the expected electrophoretic mobility was not found. Of the 15 haem contacts available for comparison, (that is residues 32, 39, 58, 62, 83, 86, 87, 91, 93, 97, 98, 101, 129, 132 and 136) 12 can be aligned to be identical to those of α chain. Of the 30 internal residues available, 22 are alike, 7 very similar and only one dissimilar to those of α chains ($\alpha 32$). Of the subunit contacts, the 16 $\alpha 1\beta 1$ contact alignments accommodate identical residues at 11 positions; the five others are 106 Leu/Gly, 107 Val/Lys, 114 Pro/Tyr, 119 Pro/Ile and 122 His/Asx. All the 10 $\alpha 1\beta 2$ contacts available for comparison are identical to those of α chains.

To determine whether substantial amounts of Hb Portland occur in normal embryos, three additional embryos below the age of 10 weeks were later obtained and from the haemolysates, separated globins were prepared, digested with trypsin and fingerprints were prepared as above. No ζ chain peptides were detected. If Hb Portland occurs in normal embryos, it must occur at a concentration well below 10%. Purified Hb Portland

is known to have a cooperative oxygen dissociation curve with a reduced alkaline Bohr effect¹⁶. If it formed 40% of the haemoglobin in embryonic cells¹⁷ it would be expected to affect their Bohr effect and cause them to differ from foetal cells rather than resemble them, as has been reported⁴. Hb Gower 1, if it is an ϵ_4 tetramer, should raise the oxygen affinity, as tetramers of like chains have very high oxygen affinities⁷. We conclude that if erythrocytes of young human embryos, aged 5–10 weeks resemble foetal cells in oxygen affinity and Bohr effect⁸ they are unlikely to contain substantial amounts of Hb Portland and Hb ϵ_4 .

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Lycorine as an inhibitor of ascorbic acid biosynthesis

LYCORINE, an alkaloid extracted from *Amarillidaceae*^{1,2}, is a powerful inhibitor of growth in higher plants, algae and yeasts. At the very low concentration of 10^{-6} M, lycorine inhibits cell division in higher plants, red algae and yeasts, and the cell cycle is arrested in the interphase stage³. No inhibition of cell division was observed in *Escherichia coli* (O. A., unpublished).

Lycorine also inhibits expansion growth in higher plants³.

At 10^{-4} M, while strongly inhibiting expansion growth, lycorine induces the following metabolic responses⁴: inhibition of ^{14}C -leucine incorporation into proteins; subsequent significant inhibition of ^3H -uridine incorporation into RNA; and a massive drop in the ascorbic acid (AA) content and a corresponding increase in dehydroascorbic acid (DHA). The effect of lycorine on protein synthesis was clearly an indirect one, as isolated polysomes were found to be lycorine-insensitive. Because RNA synthesis is only inhibited as a long term effect, it was suggested that the primary inhibiting effect of lycorine could be its action on the ascorbate system^{3,4}.

To demonstrate lycorine-ascorbic acid interaction, attempts were made to remove the effect of lycorine by exogenous administration of AA. This kind of experiment was not successful in higher plants, however, because AA was promptly oxidised by the tissue, mainly at the cut surfaces, and DHA accumulated in the cell, thus inhibiting plant growth. In contrast, the inhibition of growth induced by lycorine in red algae was almost completely overcome by the addition of external AA⁵. This was obtained because AA is very slowly oxidised to DHA on the cut surfaces so that there is no DHA accumulation in the cell, whereas AA does accumulate and thus overcomes the lycorine effect. These results show that lycorine does not interfere with AA utilisation in red algae and strongly suggest that the effect of lycorine on plants could be the result of its ability to inhibit AA biosynthesis.

To determine the effect of lycorine on AA biosynthesis, 3-d-old seedlings of *Pisum sativum*, 2 cm long and deprived of their roots, were kept in distilled water (or in lycorine, where indicated) in the dark for 18 h (starvation) and were subsequently exposed to light for 14 h. AA content of seedlings diminished during starvation: the total amount of AA+DHA decreased from 6,763 γ per g dry weight to 3,672 γ per g dry weight (Table 1). This means that a large amount of AA is utilised in cell metabolism⁶. Cells corresponding to 1 g dry weight utilise about 172 γ h⁻¹ AA in their metabolism and growth.

Actually, the amount of AA utilised in cell metabolism is higher; in fact the decrease in AA content is stronger in the presence of lycorine. With 6×10^{-5} M lycorine present during starvation, the total amount of AA is only 1,305 γ per g dry weight (Table 1). These data seem to suggest that the seedlings synthesise a certain amount of AA during starvation in the dark, as they utilise the reserve carbohydrates that are present in parenchymatous cells; this does not occur with lycorine and the AA content is consequently lowered at the end of starvation. AA biosynthesis inhibition by lycorine is better evaluated when the seedlings are illuminated after the starvation period (Table 2). In these conditions the controls synthesise a considerable amount of AA: total content (AA+DHA) rises from 3,646 to 4,776 γ per g dry weight. Lycorine present in the medium during light exposure strongly inhibits AA biosynthesis, the rate of inhibition being 41% at 5×10^{-6} M and 90% at 2×10^{-5} M. Considering that lycorine enters the seedlings only through the lower cut surface of the stem, it is very likely that lycorine concentration is quite low in the leaves where large amounts of AA are synthesised.

Table 1 Effect of lycorine on AA content in rootless pea seedlings during starvation

Time	Treatment	AA	DHA	Total (γ per g dry weight)	AA + DHA utilised in metabolism
0		5,411	1,352	6,763	—
Starvation	Water	2,800	872	3,672	3,091
Starvation	Lycorine 10^{-5} M	2,300	640	2,940	3,823
Starvation	Lycorine 3×10^{-5} M	1,910	535	2,445	4,318
Starvation	Lycorine 6×10^{-5} M	905	400	1,305	5,458

Seeds of *Pisum sativum* var. Alaska, soaked 18 h in tap water, were planted in vermiculite wetted with water, and then placed in a dark environmental chamber at 27 °C; 3-d-old seedlings, 2 cm long, were deprived of their roots and starved in the dark, at 27 °C for 18 h. The seedlings were arranged on Petri dishes through the perforated cover (25 for each dish). AA and DHA were assayed on the apices of seedlings; 50 apices for each sample, about 250 mg fresh weight, were homogenised in 8 ml 5% metaphosphoric acid and centrifuged. The supernatant was used for AA and DHA assay using 2,4-dinitrophenylhydrazine reaction^{5,10,11}. All values reported are average determinations on triplicate samples.

Table 2 Effect of lycorine on AA synthesis in pea seedlings

Time	Treatment	AA	DHA	Total (γ per g dry weight)	AA + DHA synthesised	Inhibition (%)
0		5,338	1,332	6,670	—	—
Starvation		2,782	864	3,646	—	—
14 h exposure to light	*Water	3,679	1,097	4,776	1,130	—
14 h exposure to light	*Lycorine 5×10^{-6} M	3,334	978	4,312	666	41
14 h exposure to light	*Lycorine 10^{-6} M	3,250	893	4,143	497	56
14 h exposure to light	*Lycorine 2×10^{-5} M	3,031	728	3,759	113	90

* After 18 h starvation in water the seedlings were transferred on to other Petri dishes containing either water or lycorine and placed in an aerated chamber at 1,000 lx and 27 °C for 14 h.

Table 3 Effect of lycorine on AA synthesis in potato slices

	Treatment	AA	DHA	Total (γ per g dry weight)	AA + DHA synthesised	Inhibition (%)
Fresh slices		585.8	30.2	616	—	—
Activated slices	Water	839	47	886	270	—
Activated slices	Lycorine 5×10^{-7} M	760	50	810	194	28
Activated slices	Lycorine 10^{-6} M	704	49	753	137	50
Activated slices	Lycorine 3×10^{-6} M	600	45	645	29	89

Tuber slices of *Solanum tuberosum* L. were prepared as follows: cylinders of potato tissue (0.9 cm diameter) were removed with a cork borer and sliced on a sliding microtome. Slices (1 mm thick) were washed repeatedly in tap water and these were indicated as 'fresh' slices, whereas 'activated' slices¹² were those placed either in water or in lycorine for 24 h with air bubbled into the medium. AA and DHA determinations were made on 20 slices, about 2,500 mg fresh weight for each sample, and homogenised in 12 ml 5% metaphosphoric acid. All values reported are average determinations run on triplicate samples.

Table 4 Effect of lycorine on AA synthesis in *Clivia* leaves

Time	Treatment	AA	DHA	Total (γ per g fresh weight)	AA + DHA synthesised	Inhibition (%)
0		1,584	104	1,688	—	—
Starvation		915	85	1,000	—	—
14 h exposure to light	Water	1,395	92	1,487	487	—
14 h exposure to light	Lycorine 10^{-5} M	1,410	90	1,500	500	—
14 h exposure to light	Lycorine 5×10^{-5} M	1,312	86	1,398	398	18

Young leaves of *Clivia miniata* Reg. were kept for 18 h in dark environmental chamber at 27 °C (starvation). The leaves were then placed in aerated chamber at 1,000 lx and 27 °C for 14 h. AA and DHA were assayed as indicated in Table 1, by homogenising 1 g fresh weight in 16 ml 5% metaphosphoric acid.

A demonstration of the extreme sensitivity of the AA biosynthesis system to lycorine has been provided using potato slices⁷. In contrast to pea seedlings, there are no permeability problems. In 'activated' slices, 5×10^{-7} M lycorine already inhibits 28% AA biosynthesis; almost complete inhibition is obtained at 3×10^{-6} M lycorine (Table 3).

Considering that AA biosynthesis is highly sensitive to lycorine (3 μ M lycorine inhibits 89%); that lycorine does not interfere directly with protein and nucleic acid synthesis⁴; it does not affect either permeability or phosphorylation in mitochondria and chloroplast (O. A., unpublished); it does not affect animals that are not capable of synthesising AA but does induce scurvy-like symptoms in AA-synthesising animals^{8,9}, it seems possible to conclude that lycorine is quite a specific inhibitor of AA biosynthesis in both plants and animals, and that its biological effects are probably the result of this peculiar action.

Finally, within the Amarillidaceae, results show that AA biosynthesis is much less affected by lycorine in *Clivia miniata*—an Amarillidacea containing lycorine in both leaves and rhizomes¹³—than it is in pea and potato. Lycorine (10^{-5} M), although inhibiting 56% in pea seedlings and 100% in potato slices, does not affect AA biosynthesis in *Clivia* leaves: at lycorine concentrations five times higher, the inhibition is only 18% (Table 4).

These data raise an interesting question, particularly in view

of the phylogenetic implications—what is the relationship between the AA biosynthetic pathway and the presence of lycorine in Amarillidaceae? Before any conclusions can be drawn, however, our data should first be confirmed using the isolated AA biosynthetic system.

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Intracellular killing of *Listeria monocytogenes* by activated macrophages (Mackness system) is due to antibiotic

THE classic *in vivo* studies of Mackness¹ showed that the macrophage could be activated to kill intracellular organisms such as *Listeria monocytogenes*. This led to a series of experiments to determine the process by studying killing of this organism by macrophages both *in vivo* and *in vitro*²⁻⁶. Subsequent experiments carried out with Mackness¹ system *in vitro* have required antibiotic or suitable serum in the culture medium to control extracellular growth of the organism which otherwise overruns the culture^{7,8}. Antibiotics have been shown

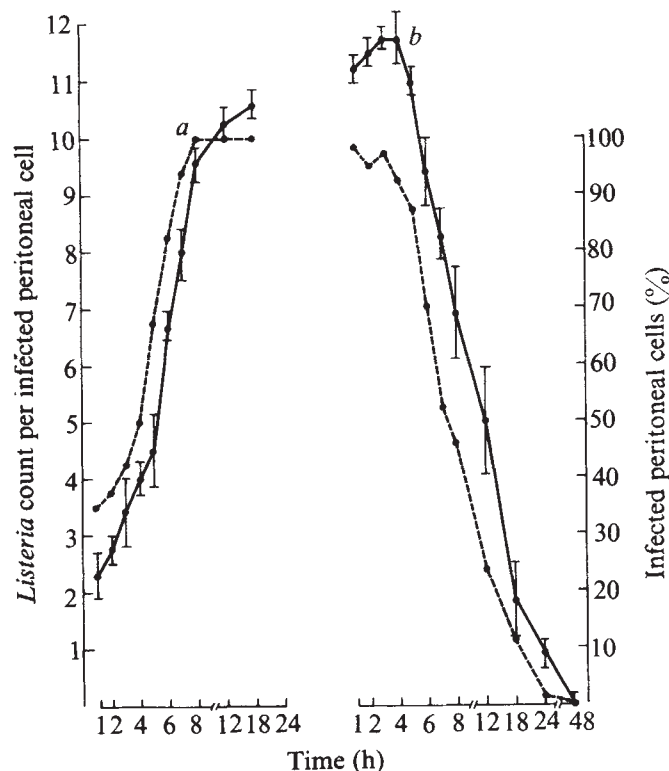


Fig. 1 The effect of antibiotic on microscopically visible avirulent intracellular *Listeria monocytogenes*. Glass-adherent cells from peritoneal washings of 10-week-old, female BALB/c mice were infected with an avirulent derivative of the *L. monocytogenes* strain NCTC5214 at a multiplicity of infection of 10. Cultures were washed and incubated in medium 199 with 20% heat-inactivated foetal calf serum in the absence (a) or presence of $0.8 \mu\text{g ml}^{-1}$ benzyl penicillin (b). ●—●, Avirulent *Listeria* count per infected peritoneal cell (left-hand vertical axis scale); ●---●, % infected peritoneal cells (right-hand vertical axis scale). Each point represents the mean $\pm$ s.d. of triplicate cultures in which 100 peritoneal cells were counted.

to enter peritoneal cells⁹ and affect bacterial proliferation¹⁰⁻¹², although there is evidence that the intracellular environment to some extent protects bacteria against the effects of antibiotics^{13,14}. It has always been uncertain, therefore, whether any intracellular killing observed has been a result of antibiotic rather than activated cellular processes. Furthermore, antibiotic might be expected to enter activated cells more than resting ones, thereby causing an apparently increased intracellular killing by the former. Thus, in order to validate the system, it is necessary to prove that antibiotic is not involved in the killing of the organism. The crucial, but hitherto absent, control that is required must show true intracellular proliferation of the organism within activated cells in the face of antibiotic in the medium.

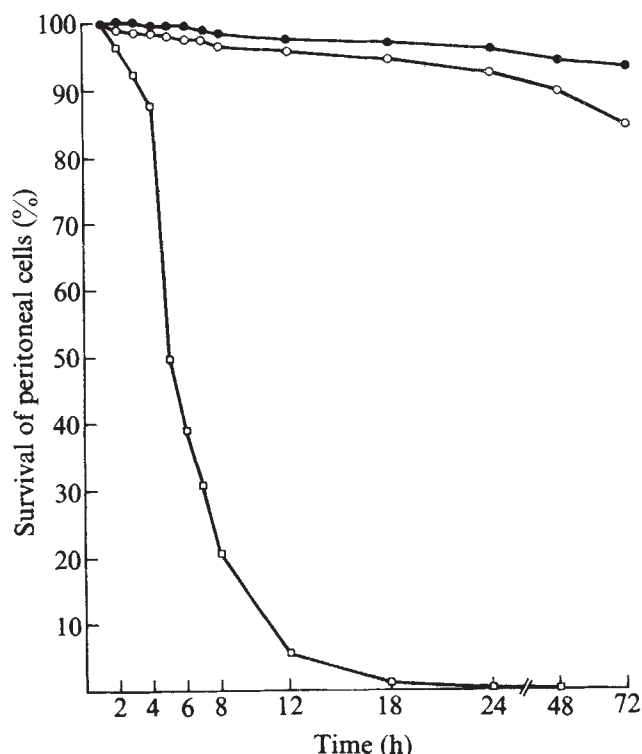
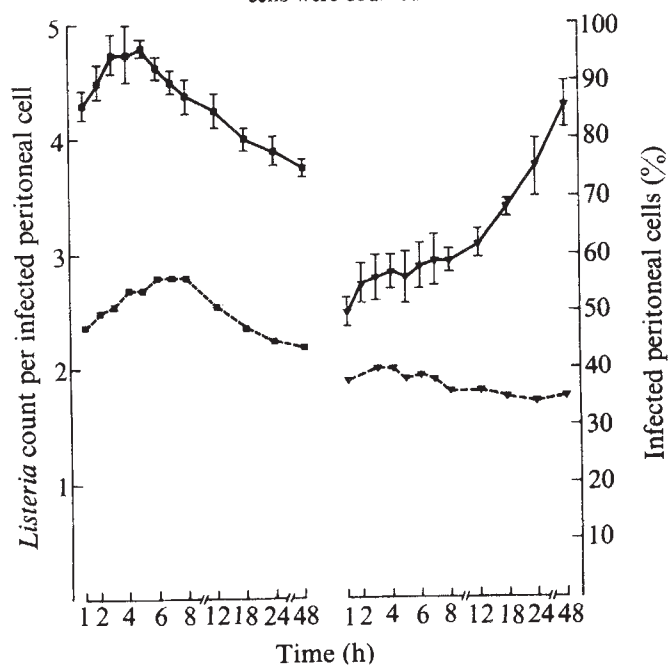


Fig. 2 The effect of antibiotic on peritoneal cell survival in cultures infected with avirulent *Listeria*. ●—●, Uninfected culture without antibiotic; □—□, *Listeria*-infected culture without antibiotic; ○—○, *Listeria*-infected culture with $0.8 \mu\text{g ml}^{-1}$ penicillin. Each point represents the mean of triplicate cultures in which 100 high power fields were examined.

In the absence of antibiotic in the medium, apparent 'proliferation' of avirulent *L. monocytogenes* within the peritoneal cells occurs (Fig. 1) and the culture undergoes premature destruction (Fig. 2). To preserve the cell culture it is necessary

Fig. 3 Microscopically visible intracellular *Listeria* in cultures with antibiotic (benzyl penicillin $0.8 \mu\text{g ml}^{-1}$). ■, *Listeria* strain NCTC5214 of intermediate virulence; ▼, virulent derivative of *Listeria* NCTC5214; —, *Listeria* count per infected peritoneal cell (left-hand vertical axis scale); ---, % infected peritoneal cells (right-hand vertical axis scale). Each point represents the mean $\pm$ s.d. of triplicate cultures in which 100 peritoneal cells were counted.



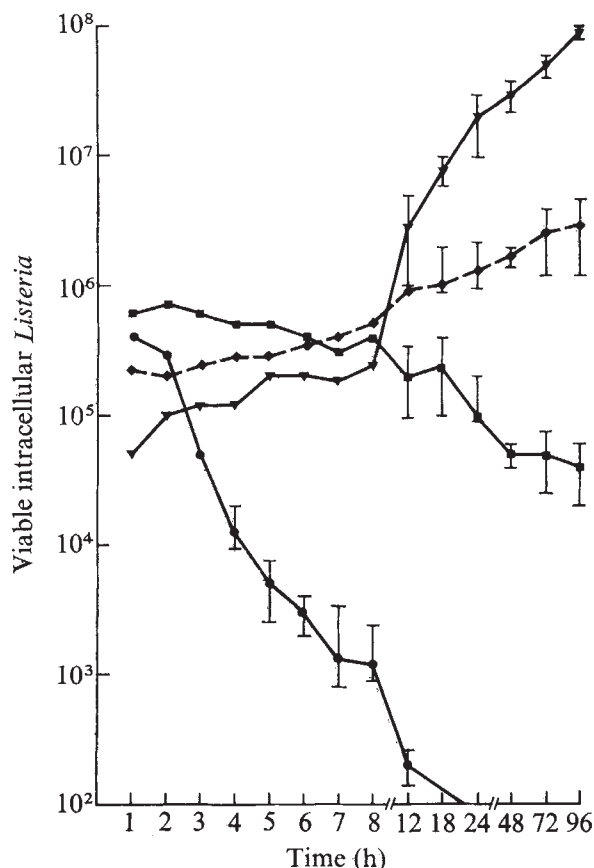


Fig. 4 Viable intracellular *Listeria* in normal and activated peritoneal cultures in the presence of antibiotic. Activated peritoneal cells consisted of glass-adherent peritoneal exudate cells induced by 2 ml of 10% proteose-peptone i.p. for 72 h. Viability assay, read at 36 h, was carried out by pour-plate dilution method in tryptose-soya agar after the cultures had been scraped with a rubber policeman, the cells lysed in distilled water and the lysate sonicated. Triplicate assays were carried out from each culture. ●—●, Avirulent *Listeria* within normal peritoneal cells; ■—■, intermediate-virulence *Listeria* (NCTC 5214) within normal peritoneal cells; ▼—▼, virulent *Listeria* within normal peritoneal cells; ◆—◆, virulent *Listeria* within activated peritoneal cells. Each point represents the mean $\pm$ s.d. of triplicate cultures.

to add antibiotic (penicillin at twice the minimal inhibitory concentration (MIC) for the organism) to the culture medium, thereby suppressing extracellular growth of the organism. This results in quite the reverse of intracellular proliferation, for both microscopy (Fig. 1) and viable intracellular counts (Fig. 4) indicate that death of the organism occurs within the cells—raising the question of whether this killing is due to antibiotic penetration of the peritoneal cell.

There is evidence for penetration of antibiotic into peritoneal cells⁹ and even for concentration of it within such cells¹⁵. It could cause *Listeria* killing within the cells in two ways. Either it could act bactericidally or it could prevent proliferation of the organism (bacteriostasis), enabling the peritoneal cells to do the actual killing. The only way to show that antibiotic does not act in these ways is to demonstrate a proper control of genuine intracellular proliferation of *L. monocytogenes* in the face of such antibiotic. Studies of strain NCTC5214 of intermediate virulence did not resolve the question. In the absence of antibiotics, strain NCTC5214 behaved identically to the avirulent derivative, but on addition of antibiotics it persisted in a viable state within the cells (Figs 3 and 4) instead of being destroyed. Nevertheless, it did not proliferate and so was not a proper control.

Proof of the ability of *L. monocytogenes* to undergo true

intracellular proliferation in the face of antibiotic in the medium was furnished by a series of experiments using a more virulent strain derived from NCTC5214 by modification of the technique of continuous passage in mice. Using this strain it was evident from microscopy (Fig. 3) and viability assay (Fig. 4) that true proliferation had occurred. Furthermore, as the MIC of antibiotic for all three strains of organism was identical ($0.4 \mu\text{g ml}^{-1}$) the different behaviour of the three strains within the peritoneal cells cannot be explained by the effects of antibiotic alone.

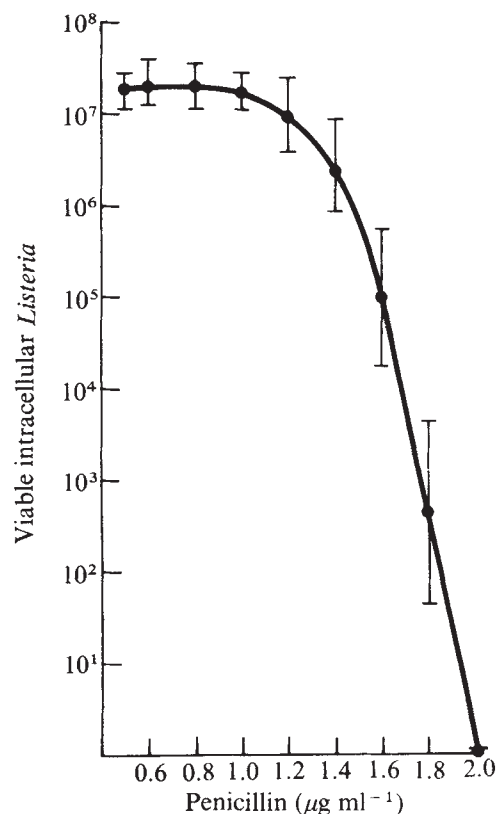
When peritoneal cells are activated by various methods their permeability to antibiotic may increase and therefore enable greater intracellular concentrations to occur. Thus, the apparent killing of intracellular organisms in the activated cell may still be due to antibiotic rather than true cellular killing.

Repeating the experiment with activated peritoneal cells and using the same three strains of *L. monocytogenes* resolved the problem. This time the behaviour of the organisms was different—the avirulent strain was killed more rapidly, the NCTC5214 strain was killed (whereas in normal peritoneal cells it had remained in stationary phase), and the virulent strain proliferated, but at a slower rate than in non-activated peritoneal cells (Fig. 4). Again, as the MIC of penicillin for these strains was identical, the increased rates of killing of the avirulent derivative and NCTC5214 strain and the slowed proliferation of the virulent strain must have been a result of factors unrelated to the antibiotic permeability of the peritoneal cells.

A dose-response experiment (collected after 18 h culture) showed that if a penicillin concentration of $1.2 \mu\text{g ml}^{-1}$ or more were achieved in the extracellular medium the virulent strain of *L. monocytogenes* was killed intracellularly. This confirms that penicillin enters the cells and can have a bactericidal effect—making a proper control mandatory.

A further problem in the interpretation of experiments previously reported is the fact that during the first 4 h of culture

Fig. 5 Dose-response effect of antibiotic on intracellular proliferation of the virulent derivative of *Listeria* after an 18 h culture. Each point represents the mean $\pm$ s.d. of triplicate cultures.



there is an 'apparent' increase in the microscopical counts of bacteria (Fig. 3) within peritoneal cells in spite of the presence of antibiotic in the medium. The viable counts (Fig. 4) do not reflect this; therefore this apparent increase is due to the phagocytosis of dead or dying organisms from the extracellular medium in the period following addition of the antibiotic at the end of the infecting phase. If only short term cultures are carried out, without close attention to viable counts of intracellular organisms, a misleading impression of intracellular proliferation may be gained.

We have shown that omission of antibiotic, because it enables uncontrolled extracellular proliferation of the organism, results in misleading data indicating more exuberant intracellular proliferation of *L. monocytogenes* than is in fact occurring. On the other hand our data show that concentrations of penicillin of $1.2 \mu\text{g ml}^{-1}$ or more kill the strain of *L. monocytogenes* used within activated mouse macrophages. As the MIC of penicillin for all sensitive strains of *L. monocytogenes* tested to date has been less than $0.5 \mu\text{g ml}^{-1}$, it is highly likely that the critical concentration of penicillin in the culture medium for most strains of this organism in this system approximates to $1.2 \mu\text{g ml}^{-1}$; but it is likely also to depend on the kind of macrophages used and (or) their level of activation. Where experiments have been carried out using higher concentrations of antibiotic it is doubtful whether activation of the macrophages *per se* has contributed to the observed killing.

The necessary control consists of true intracellular proliferation of the organism in the face of antibiotic in the medium. We have found that concentrations of penicillin less than $1.2 \mu\text{g ml}^{-1}$ enable both the intracellular proliferation of *L. monocytogenes* and the demonstration of the bactericidal property of activated macrophages.

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Electron spin resonance measurements of molecular interactions in mouse olfactory epithelium

THE mechanism by which molecules interact with, and so stimulate, the surfaces of olfactory receptor cells has given rise to much speculation. Since oil-soluble substances are often very effective odours, it has been proposed that olfactory transduction occurs when an odorant molecule perturbs the lipids of the sensory membranes. Conversely, the known steric aspects of odorant molecule interactions argue for a more specific binding for which a protein would be a more suitable receptor¹⁻⁴.

These theories are based mainly on inferences of how odorants of known chemical structure should behave in biological membranes but there is a lack of direct measurements on olfactory tissue in support of either view. Here we report the use of electron spin resonance (ESR) spectroscopy using spin-labelling techniques^{5,6} to investigate the molecular interactions of odorants at olfactory and other membrane surfaces.

Solutions of spin-labelled nitroxide compounds in saline or Ringer's (10^{-3} - 10^{-5} M) were injected into the nasal chamber of mice immediately after death by cervical dislocation or an overdose of Nembutal, and after 3-5 min olfactory tissue was removed, and placed in saline or Ringer's in an ESR tissue cell. The spectra were measured on a Varian E9 ESR spectrometer operating at the X band. Long scan times were essential to resolve the spectra. Respiratory mucosa from the nasal chamber and mucosa from the small intestine were used as tissue controls.

In the case of the four odourless spin-labelled compounds investigated using the olfactory epithelium (Fig. 1),

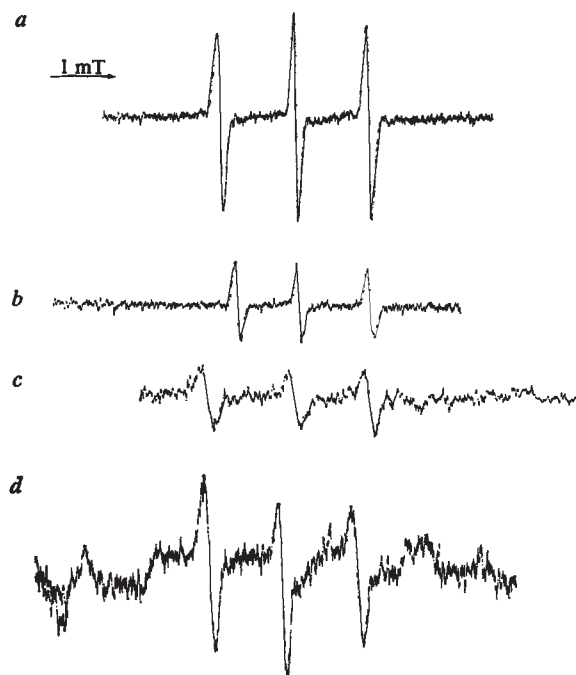


Fig. 1 ESR spectra of four odourless spin labels in the mouse olfactory epithelium. *a*, 3-Carbamoyl-2,2,5,5-tetramethylpyrrolidinoxyl; *b*, 3-carbamoyl-2,2,5,5-tetramethylpyrrolidinoxyl; *c*, 4-acetylaminio-2,2,6,6-tetramethylpiperidinoxyl; *d*, 4-hydroxy-2,2,6,6-tetramethylpiperidinoxyl.

the three amides showed spectra characteristic of their presence in an aqueous environment; since there is no evidence of molecular association with the cell membranes they are therefore assumed to remain in the mucus layer. The spectrum of the fourth spin-labelled compound (4-hydroxy) shows a splitting of the high field line (characteristic of the partitioning of a spin label between aqueous and lipid phases), and in addition a weak, strongly immobilised component.

Most of the odorous spin labels give partially immobilised spectra indicative of their solution in the membrane lipid (Fig. 2), whereas, of those compounds remaining (also the strongest odours), the isothiocyanate and *p*-toluene sulphonate ester derivatives are strongly immobilised (Fig. 3*a* and *b*), and the 4-piperidone shows a strongly immobilised component ($g=2.008$ and 1.999 ; Fig. 3*c* and *d*). Isothiocyanates readily react with free primary amino, thiol or hydroxyl groups, and in this instance, the isothiocyanate is probably so bound to mem-

brane proteins. The spin labels of each class gave spectra with the control tissues which were similar to those with the olfactory epithelium.

Previous experiments have shown a good general correlation of the odour thresholds of various mammals and of man although they cannot be claimed to be identical⁷.

Since the spectra of sensory and non-sensory tissues were closely similar it does not seem that any special features of the olfactory epithelium can be detected with this technique, but we can in broad terms draw a parallel between the olfactory potency of a compound and the extent to which it interacts with the lipids and other components of cellular membranes in general.

Recent experiments on frogs^{8,9} indicate that receptor sites on olfactory endings consist at least in part of proteins. We suggest that such proteins may be buried in the lipid of the olfactory membranes or sited at their inner surface so that an odour-lipid interaction is needed before an odour can reach the receptor site. This inaccessibility would provide a greater degree of biological specificity.

In the case of the strongest odours, a strong interaction, in some instances chemical bond formation, probably occurs between odour and membrane protein, such as to pro-

Fig. 2 ESR spectra of five odourous spin labels in the mouse olfactory epithelium. *a*, 4-Amino-2,2,6,6-tetramethylpiperidinoxyl; *b*, 4-isocyano-2,2,6,6-tetramethylpiperidinoxyl; *c*, 4-acetyloxy-2,2,6,6-tetramethylpiperidinoxyl; *d*, 4-benzyloxy-2,2,6,6-tetramethylpiperidinoxyl; *e*, 2,2,6,6-tetramethylpiperidinoxyl.

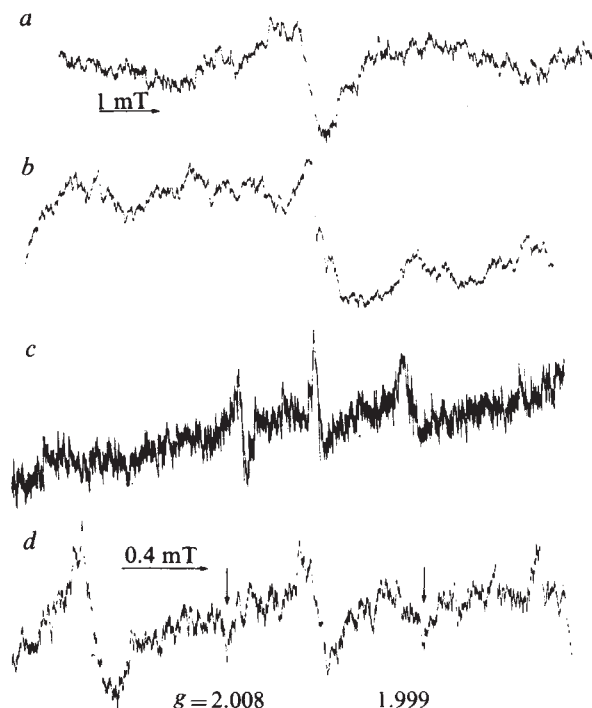
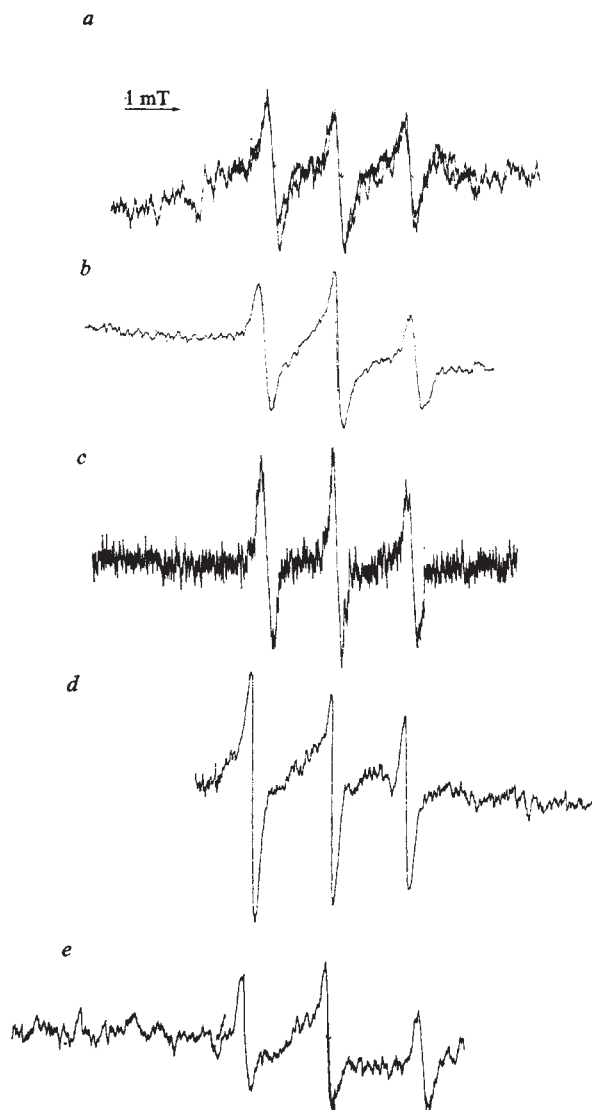


Fig. 3 ESR spectra of three pungent spin labels in the mouse olfactory epithelium. *a*, 2,2,6,6-Tetramethyl-4(4'-toluene sulphonate) piperidinoxyl; *b*, 4-isothiocyanato-2,2,6,6-tetramethylpiperidinoxyl; *c*, and *d*, 4-oxo-2,2,6,6-tetramethylpiperidinoxyl.

mote a conformational change, and so initiate olfactory transduction. Similar mechanisms may operate for the less reactive but lipophilic odours. The absence of appreciable odour in the case of the 4-hydroxy compound suggests, however, that penetration of the membrane is not in itself a sufficient condition for stimulation. This evidence therefore argues against a purely lipid-odour interaction in olfaction. Further experiments are in progress to test these conclusions, and a theoretical treatment of these results, in terms of current theories of cooperative phenomena in membranes, is in preparation.

We thank the Science Research Council for a post-graduate award to L. J. D.; and Drs Hilary Dodson and David Williams-Smith for assistance.

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matters arising

The design of wildlife preserves

MAY¹ has drawn attention to the need to make nature reserves sufficiently large, arguing from both theory and observations on island biogeography. I would like to add two comments to reinforce his case. The first is that a similar species-area relationship is found in quadrats of different size and in other studies of continuous ecosystems, as well as across sets of islands. This means that when a boundary is drawn around a nature reserve in a larger area of the same habitat, the number of species in the reserve will be less than that in the region as a whole. The other point is that if the remainder of the habitat is then cleared away, there will be an edge effect around the new reserve. Species suited to the edge will frequently be different from those suited to the centre of the reserve. If the reserve is intended to maintain the 'central' species, then its effective size is smaller than its apparent area.

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¹ May, R. M., *Nature*, 254, 177-178 (1975).

Sudden death in infancy

I AM pleased to learn that Carpenter and Emery¹ have succeeded in identifying a limited number of high-risk cases in the field of child-death prevention. But their conclusion that "there are encouraging indications that the study may be preventing some deaths" is not supported by the data.

Carpenter and Emery¹ compared a high-risk group which was not selected for follow-up health care with a sample of a low-risk group, and found that the former had significantly higher mortality. But this result merely reflects the original group assignments. The relevant issue is rather, whether or not the high-risk group which was followed up differs from the high-risk group which was not selected.

I have reanalysed Carpenter and Emery's data (Table 1) using χ -square tests corrected for continuity. Two groups (high-risk subjects not selected, and high-risk subjects either not selected or not participating) are compared separately with the high-risk group receiving aftercare. The null hypothesis for each test is that mortality will be at least as great in the follow-up group as in the non-treatment group. As statistical convention requires significance at the 0.05 level, the null hypothesis cannot be rejected in either case.

Perhaps a larger sample will yield different results in the future.

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¹ Carpenter, R. G., and Emery, J. L., *Nature*, 250, 729 (1974)

CARPENTER AND EMERY REPLY—

Magura¹ is mistaken when he says that the significant difference we reported² "merely reflects the original group assignments". The group assignments were based on a discriminant function that had been constructed before the prospective study began. The significant difference referred to is fundamental to our main point, which is that it is possible to identify babies who will be at risk of sudden death in infancy within 24 hours of their birth. We also showed that the high-risk group is sufficiently sharply defined that it can be studied prospectively.

Magura shows that the observed difference in mortality rates between the high-risk group followed up and the high-risk controls is compatible with the null hypothesis, as we were well aware. But the data are equally compatible with the alternative hypothesis that the follow-up study is preventing deaths, and on a likelihood-ratio criterion the latter hypothesis is more strongly supported than the

former. This common sense view is also reinforced by case reports³. We think that our conclusion that "there are encouraging indications that the study may be preventing some deaths" summarised this situation accurately.

We are confronted with a difficult ethical problem. To know whether or not the discriminant function and the follow-up study are effective a high-risk control group is essential. But in the light of results such as those summarised by Magura, for how long does it remain ethical to exclude high-risk babies from the follow-up study? The classical significance test approach followed by Magura gives one answer to the problem. It would be interesting to know if this view is generally shared by your readers.

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¹ Magura, S., *Nature*, 256, 519 (1975).

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Proterozoic supercontinent: time duration and the Grenville problem

THE reconstruction of the Proterozoic supercontinent has been devised¹ by superimposing 2,000-1,100 Myr palaeomagnetic poles from two regions and was derived by rotating North America anticlockwise 146° about a Euler pole at 138°E, 73°N. With this operation most dated, Precambrian, palaeomagnetic poles from Africa correlate with comparable poles from North America with fields of agreement illustrated in a qualitative way in Fig. 1. The new data of McGlynn *et al.*² enable further comparison and the 2,090 Myr Indin dykes pole agrees closely with several poles 2,070-2,090 Myr in age from Africa³. Also, 2,150 Myr poles from western Africa correlate with some poles from the Nispissing diabase and Abitibi dykes (~2,150 Myr). It is not yet possible to match 2,200-2,300 Myr poles from Africa with North American data, and there are clearly two possibilities: either the reconstruction is not valid earlier than 2,150 Myr or apparent polar movements are more complicated than recognised.

The latter explanation is probably

Table 1 Number of infants and number of sudden deaths among high-risk groups

	No. in group	No. of deaths	Significance (one-tailed test)
Followed up	354	0	
Not selected	477	4	0.15 > P > 0.10
Not selected or not participating	557	5	0.10 > P > 0.05

correct for three reasons. First, there is general agreement between pre-2,400 Myr poles on the supercontinent reconstruction (Fig. 1). This grouping seems to be definitive: the new 2,692 Myr

pole from the Dogrib dykes of North America⁴ falls in the same group and close to the 2,630 Myr pole from the Modipe gabbro of Botswana. Second, palaeomagnetic studies of the ~2,160

Myr Nispissing diabase and Abitibi dykes yield discordant poles defining similar tracks², implying rapid apparent polar movement at the time of their intrusion. Third, Archaean foliated anorthosites seem to occupy a single linear belt^{5,6} on the reconstruction.

The Proterozoic supercontinent reconstruction reconciles what has become one of the most intractable problems of North American Precambrian palaeomagnetism. Pole positions from rock units within the Grenville Province form a group practically distinct from pole positions derived from elsewhere in the Canadian Shield⁷. It is argued that the Grenville poles are essentially contemporaneous with quite different pole positions from rock units north of the Grenville Front^{7,8} and that they may be explained in terms of plate collision between the Grenville Province and the remainder of the shield at about 1,000 Myr. This explanation is difficult to reconcile with the protracted history of the province and the correlation of features across the line of the Grenville Front well into the Grenville Province (see ref. 9). The plate tectonic hypothesis has become virtually untenable in the light of new palaeomagnetic work¹⁰ yielding Grenville-type poles from a region close to the Grenville Front where there seems to be little possibility of a plate suture between the province and the shield, and also from identification of Grenville-type palaeomagnetic directions within the Canadian Shield and remote from the Province¹¹.

In Fig. 2 the Grenville Province poles from the compilation of ref. 7 are plotted on the supercontinent reconstruction where it is seen that they straddle the apparent polar wander curve recognised from African data between about 1,040 and 800 Myr consistent with abundant radiometric data from the province. It implies that the Grenville Province was an integral part of the Proterozoic supercontinent; it also suggests that this supercontinent existed as such to about 800 Myr BP.

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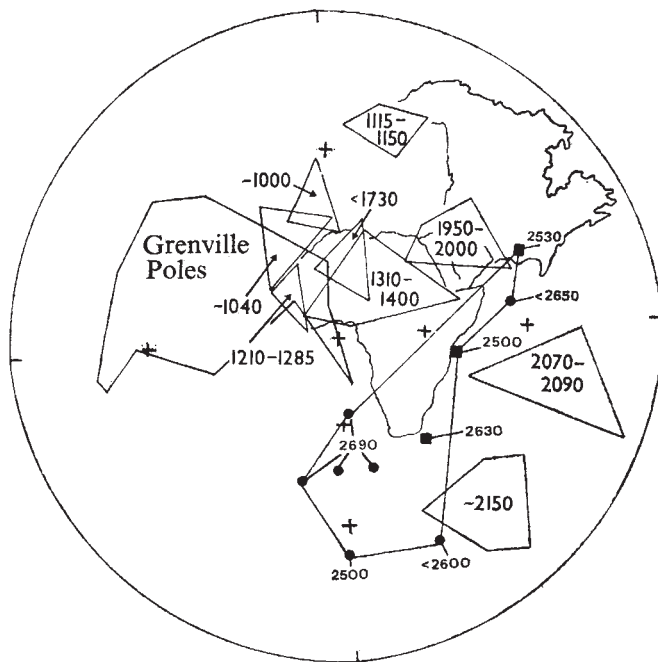


Fig. 1 Fields of agreement between African and North American Proterozoic palaeomagnetic poles on the supercontinent reconstruction. Figures give ages in millions of years. The <1,730 Myr field is defined by redating of the Mackenzie III dykes¹². Pre-2,400 Myr poles from Africa (squares) and North America (circles) are plotted and their ages indicated in millions of years.

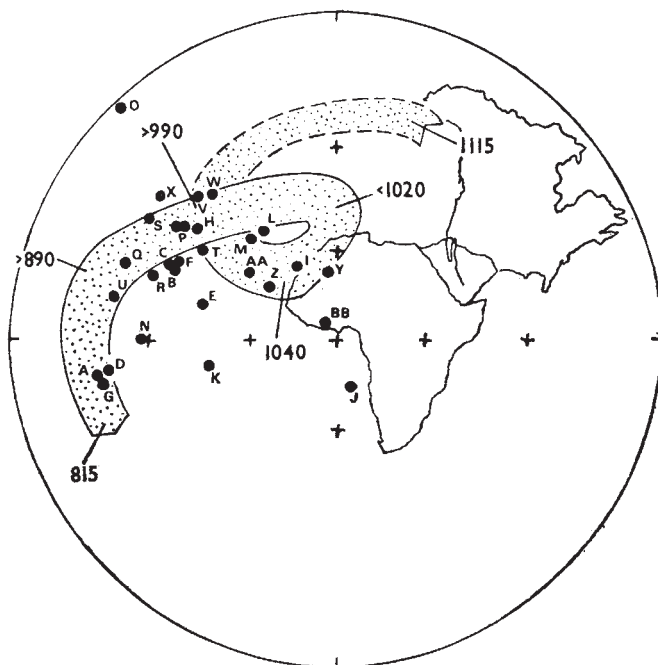


Fig. 2 Pole positions from rock units within the Grenville Province of North America plotted on the supercontinent reconstruction. The apparent polar wander path derived from African data between 1,115 and 800 Myr BP is also indicated by the stippled swathe (refs 1 and 3); this path is derived in part from stratigraphically-controlled series of poles and the figures are assigned ages in millions of years. The poles are: A, Allard lake; B, Frontenac dykes; C, Grenville gneisses; D and E, Haliburton basic rocks; F, Lake St Jean anorthosite; G and H, Morin anorthosite; I, Morin dykes; J, Magnetawan meta-sediments; K, L, and M, Mealy Mount; N, Larrimac and Bryson diorites; O, Tudor gabbro; P, Unfraville intrusive; Q, Wilberforce pyroxenites; R, S and T, Whitestone anorthosite; U, Whitestone diorite; V, Grenville front anorthosites; W, Michael gabbro; X, Seal and Croteau igneous; Y, Seal group red beds; Z, AA and BB, Shabogamo gabbro.

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Bottom life under Antarctic ice shelves

WITH reference to the article by Heywood and Light¹, it should be pointed out that the existence of a relatively rich and diversified benthic fauna under an Antarctic ice shelf was first demonstrated by Littlepage and Pearse² in 1962 for the Ross ice shelf. During November and December 1961 they collected various representatives of 16 major zoological groups (including the fish *Trematomus* sp.) using traps and grabs inserted through cracks in the shelf ice at distances of 22 and 28 km from the open sea.

As those samples included some typical 'suspension-feeders' (Porifera, Ectoprocta, Sabellida and so on), a water current able to transport the food items evidently exists and may explain the development of a rich bottom fauna under the Ross Ice Shelf. No doubt, such a current is also present under the Shelf ice of King George VI Sound, according to the shape and two openings of this ice covered body of water.

In fact, I see no reason why a substantial bottom fauna should not live anywhere under any floating Antarctic ice shelf, as many Antarctic invertebrates and fish are adapted to an opportunistic diet (including even necrophagy)³. Furthermore, there is no longer any reason to expect a peculiar (specific) fauna under Antarctic ice shelves, as it is now admitted that the Antarctic shelf has been covered by the edges of the Wurmian ice sheet. So, the main biological interest of the Ross Ice Shelf Project (RISP), when drilling at a distance of 450 km from the seaward edge of the Ross Ice Shelf, would be to obtain an insight into adaptations and relationships among the biota of these obscured areas of the Antarctic shelf.

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¹ Heywood, R. B., and Light, J. J., *Nature*, **254**, 591 (1975).

² Littlepage, J. L., Pearse, J. S., *Science*, **137**, 679 (1962).

³ Arnaud, P. M., *Third Symposium on Antarctic Biology*, Washington (in the press).

HEYWOOD AND LIGHT REPLY—We are grateful to Arnaud for drawing our attention to the paper by Littlepage and Pearse, an unfortunate oversight on our part. We note from this paper the *Trematomus* sp. was found by DeVries and Kooyman. We agree of course with the ecological comment of Arnaud. We prefer, however, to keep an open mind on whether a substantial bottom fauna can live anywhere under a floating Antarctic

ice shelf—the problems of obtaining enough food at a distance of 450 km from the open sea could be far greater than when merely 28 km away. We believe this justifies our remarks that the first biological aim of RISP is to determine whether a biome can exist at a considerable distance from the open sea, and that a biome found under ice 100–500 m thick, at least 100 km from the open sea, is "remarkable".

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Random packing of equal spheres

THE recent article of Gotoh and Finney¹ has drawn my attention to this interesting problem. I have been impressed by the amount of experimental work carried

on in this field, but at the same time I am wondering why similar experimental work has not been done—as far as I know—on the theoretically simpler problem in two dimensions to provide some evidence. I have also noticed several more or less explicit pleas to mathematicians to 'invent a statistical geometry'. None of the authors seems aware that such a geometry does indeed exist. It is usually called "integral geometry" (see, for example, ref. 2) and its methods should certainly be relevant to this problem.

The origin of integral geometry can be traced back to the famous 'Buffon's needle' problem. A needle of length l is thrown on a board ruled with parallel lines at distance d . One can show that the average number n of contacts of the needle with the lines is

$$n = (2/\pi)(l/d) \quad (1)$$

Integral geometry has several theorems such as (1) in which the constant $2/\pi = 0.6366197 \dots$ appears. Could this be the "maximum packing density" given in ref. 1 as 0.6366 ± 0.0008 and 0.6366 ± 0.0004 in two different experiments? It is certainly a conjecture worth pursuing.

In the meantime I would like to suggest an elementary explanation of the random loose packing density. Let us start from the two-dimensional case.

Circles and triangles take the place of spheres and tetrahedra. In the language of Gotoh and Finney we have a triangle—formed by an arbitrary circle and two supporting circles—which is completely specified by the angle a (see Fig. 1). To compute the packing density it is convenient to add a circle on the bottom to make the drawing more symmetrical. We can then easily see that the packing density of the configuration is the area of one circle divided by the (hatched) area of the parallelogram. Assuming a uniform probability density for the angle a , the average area A of the parallelogram is then

$$A = \frac{\int_{\pi/3}^{\pi/2} 4r^2 \sin a da}{\int_{\pi/3}^{\pi/2} da} = (12/\pi)r^2 \quad (2)$$

The limit $\pi/3$ corresponds to the case of the two supporting circles in contact; angles greater than $\pi/2$ give a configuration equivalent to one rotated by 90° . The average packing density d is then

$$d = (\pi r^2/A) = (\pi^2/12) \sim 0.8225 \quad (3)$$

All configurations being taken as equally likely with no correlations this density

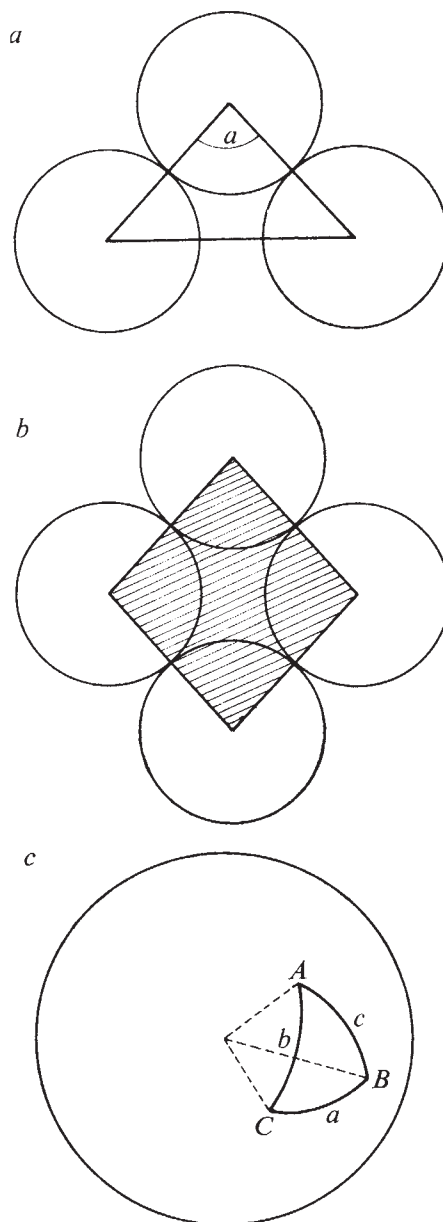


Fig. 1 Packing of circles and triangles.

should correspond to the case of random loose packing of equal circles. The three-dimensional problem can be solved in a similar way. Figure 1 should be changed into the Gotoh and Finney spheres and tetrahedron, and show a hatched parallelepiped. The three vectors from the centre of the supported sphere to the centres of the supporting spheres will then mark the vertices of a spherical triangle on a sphere of radius $2r$ (Fig. 1c). Since the probability of a configuration with b, c, A in the range $dbdcA$ is proportional to

$$\sin b \sin c dbdcA,$$

using the formula

$$\cos a = \cos b \cos c + \sin b \sin c \cos A$$

one obtains

$$\sin b \sin c dbdcA = (\sin a \sin b \sin c da db dc) / U \quad (4)$$

with $U = (1 - \cos^2 a - \cos^2 b - \cos^2 c + 2 \cos a \cos b \cos c)^{1/2}$. The volume of the hatched parallelepiped turns out to be $8r^3 U$.

The average volume of the parallelepiped is therefore (by analogy with equation (2))

$$V = \frac{8 \int_{\pi/3}^{\pi/2} \int_{\pi/3}^{\pi/2} \int_{\pi/3}^{\pi/2} \sin a \sin b \sin c da db dc}{\int_{\pi/3}^{\pi/2} \int_{\pi/3}^{\pi/2} \int_{\pi/3}^{\pi/2} \sin a \sin b \sin c da db dc / U} \quad (5)$$

With a simple change of variables ($x = \cos a$, $y = \cos b$, $z = \cos c$) we get for the density d (by analogy with equation (3))

$$d = (4/3)\pi \int_0^{1/2} \int_0^{1/2} \int_0^{1/2} dx dy dz / (1 - x^2 - y^2 - z^2 + 2xyz)^{1/2} \quad (6)$$

With a numerical integration I found $d \sim 0.596$, in excellent agreement with the experimental random loose packing density.

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¹ Gotoh, K., and Finney, J. L., *Nature*, **252**, 202 (1974).

² Santaló, L. A., *Introduction to Integral Geometry* (Hermann, Paris, 1953).

GOTOH AND FINNEY REPLY—We are aware of the numerical closeness of $2/\pi$ and 0.6366, but until now had no good nonnumerological reason for asserting its significance. We are grateful to Gamba for pointing out the possible use of integral geometry, and will follow up the

references quoted.

We stress the dangers of using two-dimensional analogues¹, for the geometries of two and three dimensions are essentially different. The two-dimensional Ising problem has been solved, although the three-dimensional one has not², and the third dimension changes the nature of percolation problems³. The average number of edges per Voronoi polygon can be shown to be exactly six for any two-dimensional array of points⁴, a fact which blurs the distinction between a two-dimensional "liquid" and crystal. The corresponding three-dimensional case is unsolved, and a discontinuous transition in the number of faces per polyhedron occurs on melting of a three-dimensional liquid⁵. Thus it is difficult to define states in two dimensions that may be equivalent to random loose or close packings in three dimensions⁶.

The simplicity of Gamba's explanation of random loose packing density is attractive, but we think it erroneous. In two dimensions, the addition of a fourth circle introduces excessive symmetry, and implicitly assumes that the covering can be considered equivalent to a covering of touching rhombuses. In a real two-dimensional non-crystalline

packing, in contrast to a dislocated solid, triangles and polygons occur. Similarly, in the extension to three dimensions, Gamba implicitly assumes that the ran-

dom loose packing can be replaced by a space-filling set of touching parallelepipeds. The parallelepiped is an octahedron with two tetrahedral caps, and immediately gives too many octahedra in comparison with the random packing. Moreover, they are distributed in too orderly a manner. The parallelepiped is bounded by six planar faces, each one being defined by four coplanar spheres. This does not fit with the reality of random packing: the extra freedom given by the third dimension leads to a low occurrence of such special arrangements, and a consequent reduction in the local symmetry within the aggregate.

The assumption of a uniform probability density for the angles a, b and c takes no account of the local geometrical restrictions on space occupation (for example, a peak near the icosahedral angle of 65° ?) although as we admitted in our original paper, we were unable to take adequate account of these ourselves. We tried to find an "average" density by the admittedly non-rigorous method of finding the "average" dimensions of an "average" tetrahedron. We tried to build in the condition that the tetrahedra, of which we try to consider the average, fill space. Thus an integral number meet at an edge, an integral number meet at a vertex, and each face is shared by two tetrahedra. In addition to these basic geometrical constraints, we also considered quasi-physical constraints, such that each sphere should make six contacts on average, and that there should be at least three contacts in any arbitrary hemisphere. Gamba, while he has taken plausible averages, does not take these or equivalent conditions into account. Moreover, by adding a similar unit to the original in a symmetrical manner to facilitate density measurement, he calculates an average over the unit cell of a primitive crystal lattice. It seems to us that his two-dimensional density is a weighted average over all possible unit cells from the square (density 0.785, $a=90^\circ$) to the hexagonal (density 0.907, $a=60^\circ$), whereas the three-dimensional value is an average over all possible primitive unit cells from the simple cubic (density 0.524, $a=b=c=90^\circ$) to the hexagonal and face centred cubic (density 0.704). We do not think this represents random packing.

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¹ Finney, J. L., *Nature*, **242**, 398 (1973).

² Stanley, H. E., *Introduction to Phase Transitions and Critical Phenomena*, 17 (Oxford University Press, 1971).

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Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

reviews

COSMOLOGY is a subject with long traditions, and general relativistic cosmology in particular has been developed in detail, both mathematical and physical, in many textbooks. In recent years there has been a distinct resurgence of interest in the subject, partly resulting from the stimulus of impressive observational discoveries (the microwave background, quasars, and so on) and partly from the elegant new mathematical techniques which have been constructed. Not least among these are the theorems of Hawking, Penrose and Geroch, which predict in very general circumstances that spacetime can develop singularities or 'edges'.

The demand for well written books covering cosmology from this new perspective is growing. Ryan and Shepley have made a useful contribution in this latest volume in the Princeton Series in Physics. An earlier volume by Peebles, called *Physical Cosmology*, deals with observational aspects, whereas this book concentrates on the mathematical side. The authors restrict themselves to models of the Universe which are homogeneous in space. These models, being a good approximation to the large scale structure of the real Universe, are simple enough to allow a tractable mathematical description, but rich enough to confront some of the more challenging and bizarre physical problems.

The early part of the book builds up the terminology and formalism of modern tensor analysis, moving straight into the Robertson-Walker isotropic

Heavenly views

P. C. W. Davies

Homogeneous Relativistic Cosmologies. By Michael P. Ryan Jr and Lawrence C. Shepley. Princeton Series in Physics. Pp. xv+320. (Princeton University Press, Princeton, New Jersey, 1975.) \$15.00.

models and the big-bang Friedmann solutions. One of the major themes of the book is then taken up with a fairly detailed description of singularities. Much of this discussion is rather sophisticated, and the new student may feel a certain frustration that very recent developments are not explained in greater detail.

Following the singularities, more traditional work is outlined on the subject of Killing vectors and isometry groups, enabling discussion of the full range of homogeneous cosmological models. Well known examples are described in some detail, though the authors have a tendency to concentrate rather too much on the more bizarre aspects. Gödel's model, which apparently allows observers to visit their own past, and the mysterious and baffling Taub-NUT and T-NUT-M spaces, each command a whole section. Some further work on singularities in homogeneous models then follows, leading naturally to the thorny subject of quantum cosmology. In Chapter 10 the authors state that "All explicitly known models which can serve as cosmological models are mathematically

singular", and point out that classical physics runs into a barrier here. The hope that quantum theory will open that barrier is long standing, but current results are still very superficial.

Some of these results are dealt with by Ryan and Shepley. They first build up the idea of 'Hamiltonian cosmology'—treating the Universe as a physical entity moving in a highly simplified fashion with a small number of degrees of freedom only. Quantisation of this simple system is then briefly alluded to. An entire chapter is devoted to the simplest of the anisotropic models, the Bianchi types I and IX, and the so-called mixmaster Universe, in which an initially chaotic motion smooths itself out into the more uniform, isotropic form observed in the real Universe today. A final chapter treats some problems in perturbation theory.

As a teaching textbook, this volume will prove useful. It is inclined to read a little breathlessly, and the level of discussion is somewhat variable, but graduate students and researchers will find it valuable for both reference and instruction. An extensive bibliography is provided, together with some exercises for the more ambitious reader. A little homespun philosophy creeps in here and there, to add a needed touch of perspective on this rather esoteric of subjects.

In summary, this book manages to get to grips with very recent results in cosmology at a time when the subject is undergoing rapid development. It is to be recommended to all serious students of modern cosmology. □

HIGH energy astrophysics is a rapidly growing field in which there have been many important discoveries, although graduate students or research workers may find it difficult to obtain a general introduction to the subject, with the possible exception of some lecture notes from summer schools. Now, however, several authors, associated with Nasa's Goddard Space Flight Center, have produced a book which may overcome this problem as far as cosmic X and γ -ray astronomy are concerned. The authors are all well known researchers, each writing about his own specialty.

About one half of the book deals with cosmic rays. Experimental techniques, results concerning nuclear and electron components, and propaga-

tion effects in space are carefully reviewed. Not much is said about sources or about acceleration mechanisms.

The other half of the book deals with X-ray and γ -ray astronomy,

High Energy Particles and Quanta in Astrophysics. Edited by Frank B. McDonald and Carl E. Fichtel. Pp. xii+476. (MIT Press: Cambridge, Massachusetts and London, 1974.) \$18.50; £9.25.

with an introductory chapter on radioastronomy. Little that is basically new has happened in X-ray astronomy over the last couple of years and these chapters can therefore be considered as a good reference. On the other hand, however,

γ -ray astronomy has recently led to the discoveries of bursts and of the SAS 2 results. Since the Goddard Center has been heavily involved in these developments, it is a pity that they were not included.

In a book of this kind a certain amount of bias is unavoidable in the choice and presentation of results. Readers curious about the more general aspects of high energy astrophysics will not find here a discussion of radiogalaxies, quasars and so on. Indeed, the authors never intended to provide an introduction to the whole field but only to those aspects indicated by the title. They have, however, certainly produced a useful collection of review articles which, largely, can be read independently.

F. Pacini

Aspects of lipid membranes . . .

Bilayer Lipid Membranes (BLM): Theory and Practice. By H. Ti Tien. Pp. ix + 655. (Marcel Dekker: New York, September 1974.) \$39.50.

SINCE its rediscovery in 1962 by Tien and his colleagues, the bilayer lipid membrane (BLM) has been used successfully in the elucidation of previously intangible problems concerning the effects of a number of substances on natural membranes at the molecular level. The value of the isolated BLM as a membrane model lies in its simplicity of composition, structure and in its intrinsic inertness. These properties allow its accurate quantitative description as a system, which is essential for interpretation of the effects of extrinsic substances.

This book is, as the author states "a highly personal account" of research in this field. Its stated objectives are to summarise the current status of the research and to present practical methods for the formation of the BLM.

Within the book the principal successes of the past decade jostle with detailed descriptions of technique; though interesting, it will be perhaps confusing for the beginner, and of doubtful value to the experienced worker. And there are several exceptions notable by their absence: nowhere is there a mention of studies of the model unit conductance channel, possibly the most important recent development in the field, initiated by Haydon and his colleagues in Cambridge in 1970. Likewise, nowhere is there a mention of the use of step functions of current and voltage to distinguish between the effects of unstirred layers, diffusion polarisation and surface properties, studies initiated by Luger and his colleagues in 1971.

In chapters 8–10, some 200 pages or so are devoted to electronic and photo-

electric phenomena. The conditions under which some of the described experiments are carried out and the abbreviations used are not explained explicitly. As these are essential in the interpretation of the results it makes for unnecessarily difficult reading.

The content of chapter 11 ranges from detailed discussions of preparations of membrane forming chemical mixtures to a description of the apparatus for measurement of fluorescence; these are plentiful in detail but lacking in principle. The "simple experiments with BLM" included in the book belie the problems likely to be encountered in practice.

All this is not to say that there is not much useful information in this unique collection of typescript writings—there is, but its value must surely rank as a limited, expensive, and rapidly dating reference book.

Edward Lea

. . . lipids in cancer cells

Lipids and Tumours. (Progress in Biochemical Pharmacology, vol. 10.) Edited by K. K. Carroll. Pp. x + 360. (Karger: Basel, London and New York, 1975.) SFr.148; £26.00; \$67.50.

THE current surge of interest in the role of membranes in homeostasis and cancer ensured that a reappraisal of the role of their lipids would follow. In this area, the fluid mosaic model proposed by Singer for the structure of animal cell membranes has gained much favour and has stimulated a number of new ideas. The model considers membranes essentially as solutions of protein in a lipid environment, the fluidity of which determines the ease with which the protein components orientate in response to a number of external stimuli. Accordingly, this review on the subject of lipids and cancer comes at an opportune time and the book will attract readers from a wider spectrum of disciplines in cell

biology than would have been the case a few years ago.

The contributors are well known in their fields and they have covered a comprehensive range of subjects including the glycerolipids, fatty acid metabolisms, proteolipids, lipids in cultured cells, fucolipids, control of lipid biosynthesis and the role of dietary fat in cancer. Unfortunately, the newer techniques of spin label and fluorescent probes are presumably too new to have been included.

The message that lipids in cancer cells are different from those in normal cells seems to be clear but the welter of changes described allows no coherent story to be formed. The use of rapidly growing 'laboratory' tumours carries with it many hazards of interpretation and since this material was used in most of the studies described it places severe limitations on conclusions about the relevance of the lipid changes to the tissue's behaviour as a cancer. Are the changes caused by the rapid growth of these cells? Which normal tissue provides the most appropriate control? Does the high proportion of necrotic tissue in some tumours affect the results? Lipidologists seem to have been slow to use the advantages of animal cell culture systems in which well controlled studies can be made with quiescent and growing normal cells, tumour cells, cells in different phases of the growth cycle, virus and chemically transformed cells and cells growing in medium containing delipidised serum with added lipids. In their chapter on lipids in normal and tumour cells in culture Howard and Howard point out the value of culture methods. An example of its application is the finding that the characteristic glycolipid change found in transformed cells in culture—the simplification of the carbohydrate moiety—is also found in rapidly growing normal cells. In the light of this result the degree to which this change is found in the range of Morris hepatomas can be interpreted as a function of their growth rate or, to put it another way, the time the cells spend in the phase of the cell cycle when the carbohydrate chain elongation is maximal—the quiescent or G₁ phase.

There is, however, much material in this book to prime the imagination of those working in the field of lipids and membranes. The format of the book and the layout of the chapter sections is pleasing. The texts read easily and most contributors have managed to avoid peppering them with references. It would have been helpful if the closing date for the literature survey for each chapter had been given and also if a short addendum with 'hot news' had been added to each section as late as possible before publication. The staggering price of this book (£26.00; \$67.50) will ensure that it will not find its way into many private libraries.

I. A. Macpherson



An Egyptian bas relief dating from 2540 BC showing cabinet makers at work (Mastaba of Tiye, Saqqara). The figure at the right is using a bow drill, perhaps the earliest forerunner of modern machine tools. From *Simple Working Models of Historic Machines*. Paper edition. By Aubrey F. Burstall. Pp. 79. (MIT Press: Cambridge, Massachusetts, and London, 1975.) n.p.

The Wild Canids: Their Systematics, Behavioral Ecology and Evolution. Edited by M. W. Fox. Pp. xvi+508. (Van Nostrand Reinhold: New York and London, March 1975.) £10.60.

UNTIL very recently no comprehensive review of the wild members of the dog family had been published since Mivart wrote his *Monograph of the Canidae* in 1890. Now two books on the subject have become available. The first, Lois E. Bueler's *Wild Dogs of the World* (Collins, London, 1974), is an excellent popular book; it is well complemented by the second, a more technical work entitled *The Wild Canids*. This latter volume is based on behavioural studies within a framework of five sections: taxonomy, behaviour, genetics, ecology, and the evolution of behaviour. Much of the work has been published before but this does not detract from the value of the book as a review.

The ethology and ecology of the northern races of the wolf, *Canis lupus*, have been studied in detail over the past decade and are well reviewed again here; and a new approach is used in a chapter on the Eskimo hunter's view of the wolf. There is still, however, a lack of any information on social structures in the Indian and Arabian races of the wolf and, as it is probable that these were the main progenitors of the domestic dog and the dingo, it would be most interesting to learn more about their social organisation (touched on by Lorenz in the foreword). Corbett and Newsome show that the dingo is not highly social and is a solitary hunter; whether this type of behaviour evolved since the dingo became feral in Australia, or whether it was inherent in their ancestors, provides a fascinating enigma to add to Macintosh's chapter on the origin of that animal.

Observations on the behaviour of the coyote, *Canis latrans*, and on some of the less well known canids, for example, the South American foxes and the Indian dhole, *Cuon alpinus*, are disappointingly slight. Lawrence and Bossert produce interesting evidence in support of hybridisation in the North American species of *Canis*. This work exemplifies an interesting aspect of canid behaviour that emerges in several parts of the book. This is the ability of certain species to have a close interaction with man (and with the domestic dog, with which the wolf, coyote, jackal, and dingo will all interbreed). Some species within the genera *Canis*, *Vulpes*, and *Dusicyon* are so flexible in their behaviour and ecological requirements that they can coexist with man and even take advantage of human disturbance to the environment.

The Wild Canids will be of great value to all students of animal behaviour. It has been well edited and the bibliography is

comprehensive. As Michael Fox says in his conclusion, one of the aims of the book is to reduce man's alienation from nature. It should help.

Juliet Clutton-Brock

Dogs, frogs and fish

Xenopus: The South African Clawed Frog. By E. M. Deuchar. Pp. x+246. (Wiley-Interscience: London and New York, February 1975.) £10.25.

A GLANCE at the list of references within this volume will indicate to the reader how rapidly *Xenopus* has risen in popularity with research workers in the field of developmental biology.

Xenopus laevis is easy to keep because it is aquatic and thus not averse to life in aquaria, even when it is kept at temperatures much lower than those normally encountered in its African habitat. The male and female can conveniently be induced to mate by the injection of appropriate gonadotrophins which are used for human medication. Dr Deuchar suggests that the sperm may need to swim faster than the sperm of other species because the male and female cloacae are not as close as they are in other anurans during amplexus. But, while collecting freshly laid eggs, I have noticed that the male partner bends his back to bring the cloacae very close when the female is actively laying eggs, but not at other times.

Most research workers concentrate on studies of embryos and tadpoles that need not be as accurately timed as mine, but staging is all important and for this they find, as Dr Deuchar says, that the *Normal Table of Xenopus laevis* Daudin is invaluable. Even a group of tadpoles that have been reared together will display differences in their rates of development, though they are smaller than the differences between groups reared at different temperatures or on diverse food regimes. It is thus rather meaningless in research to refer to the ages of tadpoles alone instead of to stages which can be quite precisely used by all investigators.

Dr Deuchar has stressed the relative speed of *Xenopus* development which may be a pleasure to some but a disadvantage to others. The beautiful grace and transparency of the *Xenopus* tadpole is a delight to all.

She has read a great deal (more than 500 references) in order to produce such a comprehensive survey of the taxonomy, anatomy, physiology and development of

Xenopus. Chapters devoted to the last of these subjects take up half of the book and provide an admirable review of present day research into many aspects of the pre-frog life of *Xenopus l. laevis*.

This book should be made available to all who work in this field. Louie Hamilton

The Early Life History of Fish. (The Proceedings of an International Symposium at Dunstaffnage Marine Research Laboratory, Oban, 1973.) Edited by J. H. S. Blaxter. Pp. x+765. (Springer: Berlin and New York, 1974.) DM98; \$40.00.

THIS volume contains the collected contributions to a symposium on the early life history of fish held at the Dunstaffnage Marine Research Laboratory at Oban, Scotland, in 1973. Fifty four papers are published in their entirety, with two more in abstract form. The volume (like the symposium) is divided into seven categories (population studies, distribution, feeding and metabolism, physiological ecology, developmental events, behaviour, and taxonomy).

Most of the contributions fall under the heading of population studies, with discussions of egg and larval abundance in relation to fish population dynamics greatly outnumbering the other papers. High mortalities during early life have a potent effect on the recruitment of a year class to a fishery and several contributions are concerned with the causes of this: the interactions of starvation, predation, physical damage, loss to adverse current systems, and imperfections in development, are all discussed. Clearly, the causes are varied and rarely stem from any one factor.

The section on physiological ecology contains several valuable papers on environmental topics. The effects of cadmium on the development and survival of herring eggs, and the effects of thermal shock on larvae entrained in electricity generating station cooling systems, are two papers of particular relevance to fisheries in industrial regions. Another contribution discusses the effects of H_2S on the development and survival of larvae in freshwater fishes.

Under the heading 'Rearing', six papers cover techniques related to aquaculture. Variation in temperature and in photoperiod, hormone injection, selective hybridisation, and genetic manipulation all have an obvious relevance to rearing fish for food and are considered in various contributions.

These wide-ranging topics are typical of the broad spectrum of research into fish eggs and larvae that was presented at the symposium. The editing of this volume (and the organisation of the meeting) provides a lasting testimony to J. H. S. Blaxter's enthusiasm for this specialised field. Alwyne Wheeler

obituary

Maurice Freedman, a leading social anthropologist, died on July 14 at the age of 54.

Professor Freedman followed a distinguished career in London by becoming head of the Institute of Social Anthropology at Oxford and a Fellow of All Souls and at the time of his death was Professor of Social Anthropology at Oxford. He had entered the department of anthropology of the London School of Economics in 1946, his first work involving studies of racial and cultural relations in Malaya. Appointed to a lectureship in anthropology at the LSE in 1950, he became reader in anthropology in the University of London in 1957, and was elected to a personal chair of anthropology in 1965—moving in 1968 to an established post in this subject. But his main interests lay in the field of overseas Chinese society. In 1949–50 he spent nearly two

years of field research among the Hokkien-speaking Chinese of Singapore, publishing as a result *Chinese Family and Marriage in Singapore* in 1957. For the next 25 years, he was to build on this framework to form a deep understanding of basic Chinese concepts and institutions. He clarified the structure of lineage and kinship in south-eastern Chinese society; the nature of ancestor worship; and the significance of geomancy—the art of relating a site, especially for burial, to the good fortune of men. Professor Freedman was also deeply concerned about the position of Jews in the social life of Britain.

Carl O. Sauer, who was emeritus professor of geography at the University of California at Berkeley, has died at the age of 85.

Sauer was for more than 30 years chairman of the geography department

at Berkeley, retiring in 1957 as emeritus professor. He did much to establish Berkeley as a school of "cultural geography". He established the department's doctoral programme, and became a leading and widely published authority on desert studies, tropical areas, the human geography of Indian populations, and agriculture and native crops of the New World. He maintained that all geography is essentially historical. Written documents, archaeological evidence, plant data, soil profiles—all provided him with clues for unravelling problems of the human global environment. His many awards included the Victoria Medal from the Royal Geographical Society, which he received recently, the Vega Medal from the Swedish Society for Anthropology and Geography in 1957, and the Charles P. Daly Medal from the American Geographical Society in 1940.

announcements

Awards

J. Bingham has been awarded the **Mullard Award** by the Royal Society for his contribution to agricultural productivity by breeding a series of winter wheat varieties.

The Potato Marketing Board has awarded a **James E. Rennie Award** to **D. N. Crowe** and **J. A. H. Taylor**.

Appointments

F. G. T. Holliday has been appointed a member of the Oil Development Council for Scotland.

The Nature Conservancy Council announce that **F. B. O'Connor** has been appointed deputy director.

International meetings

September 3–5, **Child language**, London (The Information Officer, School of Oriental and African Studies, Senate House, Malet Street, London WC1E 7HO, UK).

Person to Person

Bibliography on reconstruction from projections. Listings needed in computerised topography, radio astronomy map synthesis, multi-dimensional profile inversion, 3D electron microscopy, zeugmatography, inversion of Radon transform, and so on, all problems in reconstructing 2D and 3D images from data obtained by projection or line integration. To be published. (Richard Gordon, Image Processing Unit, National Cancer Institute, NIH 36/4D28, Bethesda, Maryland 20014).

There will be no charge for this service. Send items (not more than 60 words) to Holly Connell at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

September 9–10, **Morphology and biology of living and fossil reptiles**, London (The Executive Secretary,

The Linnean Society, Burlington House, Piccadilly, London W1V 0LO, UK).

October 1–10, **High energy radiation dosimetry and protection**, Sicily, Italy (Dr A. Rindi, Building 72, Room 122, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720).

October 6–8, **Fertility and sterility**, Madrid, Spain (Professor E. V. Dominguez, Fernal Pardines 82-c-70 DCHA, Madrid, Spain).

Miscellaneous

Neuberger report. The British Nutrition Foundation has decided to institute an essay competition on 'Nutrition is an emerging subject; discuss in relation to the Neuberger report'. The competition (essay length up to 2,500 words) is open to young scientists and medical students under 25 and prizes of £100, £75 and £50 will be awarded. Entries (before December 31, 1975) to: Mr P. M. Victory, Secretary, British Nutrition Foundation, Alembic House, 93 Albert Embankment, London SE1 7TY, UK.